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THE STAINING OF ACID-FAST BACILLI. THE EFFECTS OF FIXATIVES AND H-ION CONCENTRATION ON ACID-FASTNESS

by

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(Received for publication 1st February, 1934.)

According to Churchman, the discovery of the phenomenon of acid-fastness was made by Neisser in 1881 in his application of Weigert's staining methods to the leprosy bacillus. Ehrlich (1882) made a similar observation regarding *Mycobacterium tuberculosis*, using a mixture of a basic dye and aniline water on the assumption that tubercle bacilli possessed a membrane which was penetrated only by an alkali. He regarded aniline as a substitute for potassium hydroxide, and found that specimens so stained could be treated with strong hydrochloric acid without extracting the stain. Ziehl (1882) opposed the views of Ehrlich regarding the alkaline reaction of the staining fluids, and suggested the use of phenols; he also used pyrogallic acid and resorcin with equally good results. Ehrlich tried carbolic acid, and concluded "that it was a less perfect substitute". Neelsen's (1883) suggestion that fuchsin should replace the methyl-violet of Ziehl was adopted, and the method is now known as Ziehl-Neelsen's.

It is well known that acid-fast bacilli vary in the degree of intensity with which they take up the stain, and that when present in tissues preserved by formalin or other fixatives they may fail completely to hold the stain.

The present investigations have been carried out in order to explain, and find a method to correct, the loss of acid-fast staining properties in bacteria.

ZIEHL-NEELSEN STAINING AFTER THE USE OF FIXATIVES.

Haythorn (1929) states that, if sufficient time is allowed, formalin will cause the bacilli to lose completely their acid-fast properties; he also maintains that re-staining is a failure.

We have examined smears from tissues which had been fixed and stored in ordinary 10 p.c. formalin for periods up to 20 years. The results indicate that, although the acid-fast property of the bacilli is inferior, it is by no means destroyed, the organisms showing when stained by Ziehl-Neelsen 25 to 50 p.c.

acid-fastness, and when stained by alkaline fuchsin, 100 p.c. fastness. Tissues fixed for 6 to 7 years in either Kaiserling, Craig, or Klotz fixatives (alkaline formalin solutions) showed acid-fastness from 50 to 100 p.c., the higher percentages being registered after fixation in Klotz, when the final pH values were higher and on the alkaline side of neutrality. Murray (1924), by soaking his material in ammonia prior to staining, obtained good staining results with Ziehl-Neelsen.

Tuberculous tissue of avian origin, which had been fixed and stored for 1½ to 3 years in methylated spirit, when stained by Ziehl-Neelsen, gave negative results on examination; but when stained with alkaline fuchsin gave maximum acid-fastness.

Zenker and von Rath's fixatives do not have such a destructive influence on the staining properties of acid-fast organisms. Although Haythorn found that Zenker-fixed specimens showed some reduction, he concluded that as a fixative it was superior to formalin. We have found that a certain variation in density of staining occurs after Zenker. Fixation of material in von Rath's picro-sublimate gives more consistent results than either Zenker or formalin. As these fixatives contain acetic acid and picric acid respectively, prolonged treatment was given similar to that obtainable with formalin fixation, and a detrimental effect was noted on the staining of the organisms. Heating smears in 1 p.c. acetic acid and concentrated picric acid at various temperatures from 37°C. to 100°C. had a pronounced destructive effect, varying more or less with the temperature. When smears were subjected to boiling in 1 p.c. hydrochloric acid and then stained with Ziehl-Neelsen no organisms could be found; similarly no organisms were found when smears were boiled in Zenker with or without acetic acid (fresh or old solutions). Smears could be boiled for an indefinitely prolonged period in solutions of sodium carbonate either alone or mixed with formalin or Zenker solution (minus acetic acid) without interfering with the acid-fast qualities. The superiority of both the above fixatives over formalin, therefore, is, we suggest, not due to their inherent qualities, but rather to the fact that (a) smaller pieces of material are fixed, and (b) greater care is taken in the preparation, with a quick transition to the cutting stage.

ASSOCIATION OF pH VALUES AND DESTRUCTION OF FASTNESS.

Loeb (1922), using a fuchsin-gelatin mixture at values above pH 4·7, found that the colour was permanent, but below this figure the colour was absorbed. Briggs and Bull (1922) brought forward evidence to show that the taking up of stains took place by adsorption, which varied with the pH value of the solution.

After carrying out a long series of observations on tissue fixed in formalin or alcohol, we concluded that the factor responsible for the apparent destruction of acid-fastness was not the fixative, but changes brought about by auto-

lytic action in the tissue. These changes are directly responsible for lowering the pH value of the fixing solution. Bradley and Taylor (1916) found that autolytic changes were responsible for a change from an alkalinity of pH 7·4 to an acidity of pH 6·0. The destruction of staining power in sections is accentuated by treatment with fluids having an acid reaction, such as alcohol, xylol, balsam, etc., which are frequently used for lengthy periods. The tissues, moreover, besides being in close association with fluids having low pH values, are brought finally into contact with a carbolic acid stain, which has a pH value of 2·0 (Bogen's indicator). Changes in the fixing solutions undoubtedly take place in the early stages of fixation. We have noted such changes repeatedly in museum jars, and invariably a final pH value of 4·0 or even less has been recorded for formalin and methylated spirit. The specimens in formalin had been stored up to 20 years, and in spirit up to 3 years. Estimations have been carried out with bulky tissue placed in 10 p.c. formalin supersaturated with sodium bicarbonate, showing an initial reaction of pH 8·0 (Bogen's indicator). Autolytic action was responsible for a steady change in the reaction of the fixing solution. In one case, after 49 days, it was lower than pH 4·0. On the other hand, a smaller amount of tissue was fixed in the above solution heated to boiling point, and when left exposed for the same period showed no appreciable change in reaction.

EFFECT OF FORMALIN AND ACETONE ON ACID-FAST ORGANISMS.

Formalin and acetone as destroyers of acid-fastness have also been suggested. Dreyer (1923) states that tubercle bacilli, heated to 100°C. in 40 p.c. formalin, and then extracted in acetone at 65–70°C. for twenty to twenty-four hours, lose their acid-fast and Gram-positive characters. In view of our previous findings it was considered advisable and necessary to repeat Dreyer's work, substituting tissue smears for his culture material and taking precautionary measures regarding the reaction of the solutions.

The solutions were kept on the alkaline side with additions of small quantities of sodium bicarbonate or ammonia, brom-thymol-blue being used as the indicator in the control of the reaction. Boiling of smears was carried out in flasks fitted with condensers, either on an electric hot plate or a water bath. After each step in the procedure a slide was taken from the flask and stained with the alkaline fuchsin mixture.

When smears containing numerous tubercle bacilli were treated for extended periods, irregularly alternating with alkaline formalin and acetone, both at boiling temperature and cold, there was no destruction of acid-fast properties of the organisms.

In our observations the smears receiving maximum treatment [(alkaline formalin 16 hours at 100°C. and 54 hours at room temperature) plus (alkaline acetone 45 hours at 68°C. and 156 hours at room temperature)] were equally as good in their staining quality as those smears receiving intermediate treatment [(alkaline formalin 4 hours at 100°C. and 36 hours at room temperature) followed by (alkaline acetone 4 hours at 68°C.)] or smears receiving no preliminary treatment.

It would appear, then, that the factor responsible for the apparent destruction of acid-fastness in tissues fixed in formalin or alcohol is also responsible for the destructive influence noted by Dreyer. It would appear also that the fluctuating results obtained by this author were due, especially in the case of the formalin, to a variation in the acidity of his solutions.

In the present series of experiments, in which the solutions were kept definitely on the alkaline side of neutrality, no destruction or fluctuation of acid-fastness was observed.

It is well recognized that samples of formalin vary in their reaction, and to a lesser degree acetone is open to the same objection; and Dreyer does not appear to have controlled the reaction during the treatment of his material.

FIXATION AND STAINING.

Treatment of tuberculous and leprous material was carried out by two methods, both giving excellent results.

(1) Fixation of small pieces of tissue in 10 p.c. formalin containing 0·5 p.c. sodium bicarbonate (stain with Ziehl-Neelsen).

(2) Staining tissue fixed in formalin or alcohol with an alkaline fuchsin mixture.

This alkaline fuchsin method has been designed to overcome the poor staining results obtained with Ziehl-Neelsen, when tissues are fixed in formalin or alcohol. The stain should be made up in two solutions which are mixed immediately before use in the proportions of 1 part of solution A to 3 parts of solution B.

Solution A. Sodium bicarbonate 0·25 p.c. concentration.

Solution B. Basic fuchsin 1·5 gm. dissolved in 40 c.c. of absolute alcohol made up to 400 c.c. with tap water, having a reaction of about pH 8·0.

Staining was carried out by steaming for three minutes with the mixture, or as an alternative bringing the mixture first to boiling point, pouring it on the slides and staining for at least ten minutes. The slides were then washed in running water and decolourized for two minutes in 15 p.c. sulphuric acid or other decolourizer, again washed well and counter-stained with 1 p.c. methylene blue for two minutes. Sections were further carried on to the dehydration and clearing stages and mounted in neutral or slightly alkaline Canada balsam.

It is interesting to note that a comparison of the relative value of the alkaline treatment as compared with formalin having a slightly acid reaction reveals evidence in favour of the former. Lung tissue from a monkey containing numerous acid-fast organisms was fixed in ordinary 10 p.c. formalin for one month. From this, sections were cut and mounted on slides which were divided into two series, one of which was stained immediately with Ziehl-Neelsen, washed, and placed in 15 p.c. sulphuric acid. At intervals the slides were taken from the acid, washed, counterstained, and mounted in balsam. An examination of the sections after sixty-six hours' immersion in the acid revealed a loss of staining property equal to about 80 p.c. The second series of specimens was placed in 0·5 p.c. sodium bicarbonate solution for two weeks. These slides were then stained with Ziehl-Neelsen, washed, and placed in 15 p.c. sulphuric acid. At intervals they were taken from the acid, counterstained,

mounted, and examined; after two weeks in the acid, the bacilli showed fully 100 p.c. staining and acid-fastness. By the end of the fourth week a loss of only 25 p.c. of the staining of the bacilli was noted, while at the end of ten weeks a loss of 50 p.c. was recorded. At fourteen weeks a further reduction had taken place, but even after this prolonged immersion in the acid solution the staining of the bacilli was slightly superior to that in the first series after only sixty-six hours' immersion in acid.

SUMMARY.

1. Neither formalin nor alcohol used as a fixative is responsible for the permanent destruction of acid-fastness (bacillary).

2. Neither formalin nor acetone, nor treatment with both, is responsible for the destruction of acid-fastness.

3. Ehrlich's original observation regarding the necessity for alkaline treatment of acid-fast bacilli would appear to be sound.

4. Autolytic action in tissues is responsible for lowering the pH value of the fixative. Low pH values are responsible for the change in the staining reaction of acid-fast organisms.

5. The staining difficulty may be overcome by controlling the pH value of the fixative, keeping it on the alkaline side; or by staining with an alkaline fuchsin.

6. A new method of staining formalized tissue is given.

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THE YIELD AND COMPOSITION OF THE MILK OF THE MERINO EWE

by

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INTRODUCTION.

There appears to be little information available concerning either the yield or the composition of the milk of the Merino ewe. As far as the author is aware the only published work is that of Fuller and Kleinheinz (1904), who determined both yield and composition of the milk of six ewes, one of which was a Merino. This animal, which had lambed at least seven weeks previously, gave 880 gm. of milk per day over the experimental period of 48 hours. The specific gravity was 1·0395, the content of fat 6·45 p.c., and the total solids 17·615 p.c.

More observations have been published concerning the milk of the mutton breeds of sheep. Ritzman (1917) determined the content of fat of 158 samples from 95 ewes. These animals were drawn from six different breeds and eleven crosses between them. A greater variation was noticed between the percentages of fat of the milk of individuals of each breed than between the averages for the different breeds. The average content of fat for the milk of all the sheep was 6·0 p.c. The averages for sheep of different ages from 2 to 7 years were almost identical. Actual milk yields were not measured, but Ritzman was able to group the sheep in four classes, according to the amount of milk which he estimated they were producing. He found that the rate of growth of the lambs increased with the yield of milk of their mothers. Neidig and Iddings (1919) studied the yield and composition of the milk of three ewes from each of six mutton breeds. Determinations were made at intervals of 10 days from the tenth to the fiftieth day of lactation. The average daily production over the entire period of observation varied from 2·17 kg. for the Hampshire sheep to 1·24 kg. for the Southdowns, the average for all breeds being 1·54. The average for all breeds on the tenth day, when the maximum yield was obtained, was 2·01 kg., while on the fiftieth day it was 1·21 kg. Specific gravity showed a variation for the different breeds of 1·027 to 1·036, with an average of 1·032.

The variations and averages for the percentages of the various constituents were: Casein, 3·06–3·60, 3·29; albumin, 0·74–0·81, 0·77; non-protein N, 0·007–0·078, 0·059; fat, 7·1–8·1, 7·7; lactose, 4·45–4·76, 4·65; and ash, 0·76–0·91, 0·83. The authors attempted to compare the growth of the lambs with the total quantity of the constituents of the milk which their mothers produced, but this comparison was complicated by the different numbers of lambs in the various groups.

Much information is available concerning the milk produced by the various breeds of milk sheep of Europe. Weiser (1920) published the results of his observations on the yield and composition of the milk of two Friesian ewes, at intervals of one month, for a period of six months. The yields at the first determination in April, apparently the second month of lactation, were 1·50 and 1·35 litres respectively; the amounts fell steadily until, in September, they were 0·30 and 0·20 litres respectively. The average specific gravity for the whole period was 1·037, and the average percentage composition was: Dry matter 17·10, fat 6·24, lactose 4·29, casein 4·52, albumin 0·92, amide 0·30, and ash 0·85. Weiser tabulated the results obtained by a number of workers concerning the composition of the milk of various breeds of milking sheep. These results were in close agreement with his.

In order to supply some information concerning the milk of the Merino ewe the present work was carried out during the lambing season of 1933. It was hoped to measure the total yield and determine the composition of the milk at three stages of lactation, but actually it was only found to be possible to carry out the determinations at two different periods.

THE EXPERIMENT.

Six Merino ewes whose ages varied from 2 to 6 years, and all of which had lambed during the same week, were selected for the experiment. As far as could be judged from the growth of their lambs these ewes were producing a normal amount of milk. The ewes grazed throughout their lactation period on natural pasture, consisting chiefly of annual species.

Observations were made during the third and ninth weeks of lactation.

The lambs were separated from their mothers throughout the duration of the experiment, except when actually suckling. After having been away from their mothers for two hours the lambs were allowed to suckle. During the next 48 hours the lambs were allowed to suckle at intervals of 1½ hours throughout the daytime in the first experiment, and at intervals of 2½ hours in the second period, being weighed before and after each suckling. By this arrangement the lambs were able to suckle seven times per day in the experiment in the third week of lactation, and five times daily in that of the ninth week.

Samples were drawn from the ewe at alternate milkings, just before the lamb was allowed to suckle, the amount taken being roughly proportional to

the quantity of milk given by the ewe at that milking. The various samples from each ewe were mixed, and the composite sample was preserved with one drop of formalin per 100 c.c. of milk and kept in a refrigerator at 0–5°C. until analysed. While it was realized that, in the case of the cow milked twice daily, "fore milk" has not the same composition as that obtained in the middle of the milking or that of the "strippings", it was believed that the use of either five or seven milkings per day would greatly reduce this error.

METHODS OF ANALYSIS.

Milk for analysis was weighed either in a covered beaker or dish or in a weighing bottle. The following methods, taken largely from Allen (1932) were used:

Specific gravity: Determined by means of a specific gravity bottle.

Total solids: 3 or 5 c.c. were dried in a silica dish on the water-bath for 30 minutes, and then in an electric oven at 100°C. for 2 hours; cooled and weighed; dried and weighed until constant in weight.

Ash: 25 c.c. were dried in a silica dish and then ashed in an electric muffle below a dull red heat. Heated and weighed until constant in weight.

Total nitrogen: Determined by the Kjeldahl method on 5 c.c.

Casein: 10 c.c. were diluted with 50 c.c. of water at 40–42°C., and casein precipitated at the iso-electric point, pH 4·7, by addition of N/10 acetic acid until approximately the correct pH was reached, using brom-cresol-green, then by addition of excess of an acetic acid-sodium acetate mixture (half-neutralized acetic acid) having a pH of 4·7; filtered, and n nitrogen determination made on the precipitate. The factor used was 6·38.

Lactalbumin: Sufficient 20 p.c. trichloracetic acid was added to the filtrate from the casein to give a concentration of 4 p.c.; heated on the water-bath for half an hour, and filtered. Nitrogen estimated on the precipitate, factor 6·38.

Non-protein nitrogen: Obtained by subtracting the sum of the casein N and lactalbumin N from the total N.

Fat: 5 c.c. were absorbed by a coil of filter paper, dried at 100°C. and extracted with ether in a Soxhlet for 5 hours.

Lactose: 7·5 c.c. milk were placed in a 150 c.c. volumetric flask, diluted with water; $CuSO_4$ and NaOH added to precipitate proteins, made up to the mark and filtered, the filtrate being still acid and containing copper in solution; lactose was determined on an aliquot of the filtrate with Fehling's solution, the precipitated cuprous oxide being filtered on a Gooch crucible, heated in an electric muffle, and weighed as cupric oxide.

Calcium: The ash was dissolved in dilute HCl, and calcium determined on an aliquot by precipitation as oxalate by the usual procedure, and finally titrating with $KMnO_4$.

Phosphorus: Determined by the colorimetric method of Fiske and Subbarow (1925) on an aliquot of the ash solution.

Energy: Determined on milk solids by combustion with oxygen in a bomb calorimeter.

RESULTS.

The daily yield, specific gravity, energy, and composition of the milk of the individual ewes at the third and ninth weeks of lactation, together with the average values, are set out in Table 1.

DISCUSSION.

The animals used in this experiment were the large-framed Merinos from the Middle-North of South Australia. Although sheep of this type attain a larger size than Merinos from other localities in Australia, their rate of growth during the first few weeks of life does not appear to be greater. Thus, lambs from the ewes employed in this work doubled their weight in 10 to 21 days from birth, the average time being 17 days. Figures from various parts of Australia show that Merino lambs double their weight in 2 or 3 weeks from birth. It would appear, therefore, that the results obtained here should apply to the milk of the Australian Merino in general.

When the first set of determinations was made, that is, during the third week of lactation, the average daily yield of milk was 1,205 gm. This is about two-thirds of the value obtained by Neidig and Iddings (1919) with ewes of mutton breeds at a similar stage in lactation. Weiser (1920) did not determine the yields from his milking sheep until the second month of lactation. However, inspection of his figures for yields over six months suggests that the daily production of milk in the first month was similar to that of the mutton breeds at a corresponding period in lactation. The percentage of casein in the milk from the Merino was about 10 p.c. higher than for mutton breeds, but about 20 p.c. below that for milk breeds at a similar period in lactation. The percentage of fat was about the same as for the mutton breeds of Neidig and Iddings, but about 12 p.c. higher than that of the sheep observed by Ritzman (1917), and about 50 p.c. higher than that of Weiser's milk sheep. Albumin, lactose, and ash had similar values in all three types of sheep.

The total yield of milk in the ninth week was only 50 p.c. of that in the third week, during which, probably, the maximum yield was obtained. From Neidig and Iddings' work it appears that the maximum yield for individual sheep lay between the tenth and the twentieth day. For all the sheep the yield on the tenth day was the greatest that was observed. The yield on the fiftieth day was only about 60 p.c. of that on the tenth day. A similar decrease is seen in the results published by Weiser. Coincident with the decrease in total yield there was an increase in the Merino's milk of 9 p.c. in total solids. This was due chiefly to the increase in casein, amounting to 28 p.c., and also to an increase in fat of 7 p.c. Phosphorus also increased, and was largely responsible for the increase in total ash. Lactose, albumin, and calcium remained almost unchanged. Energy increased with the increase of organic matter. Neidig and Iddings noticed an increase in casein but a decrease in fat and lactose with advance in lactation, and Weiser an increase in casein, fat, and ash, with a decrease in lactose.

It was not found possible to compare the growth of lambs with either the total quantity of milk or of any constituent, owing to the small number of

animals used and to the diversity of their birth weights, which varied from 3·6 to 5·8 kg.

It is interesting to note that the energy, as determined, approximated to that calculated from the formula drawn up by Overman and Sanman (1926) for computing the total energy of cow's milk from its composition. In the case of the ewe's milk the calculated values in calories per U.S. quart were 1,107 and 1,211 respectively, at the two stages of lactation, whereas the values actually found were 1,108 and 1,196 calories.

At the time of the first series of determinations the lambs were deriving almost the whole of their energy from the milk of their mothers. The gross energy of the milk at this time averaged 1,360 calories per day. It has been found by Peirce (1933) that the basal metabolism of lambs 3 to 12 days old is 1,850 calories per sq. metre of body surface per day. At 18 days old it would have a lower value. The average weight of the lambs was 10 kg., equivalent to a surface area of 0·47 sq. metres. The basal metabolism of these lambs would therefore be under 850 calories per day.

SUMMARY.

The average daily yield of milk from six Merino ewes was 1,205 gm. during the third week of lactation and 650 gm. during the ninth week. The average percentage compositions at the two stages were: Total solids, 17·66 and 19·29; casein, 3·43 and 4·38; lactalbumin, 0·81 and 0·85; fat, 7·41 and 7·90; lactose, 4·83 and 4·81; ash, 0·86 and 0·90; phosphorus, 0·095 and 0·13; and calcium, 0·20 and 0·21. The gross energy was 1·13 and 1·22 calories per gm. respectively at the two stages.

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I desire to thank Mr. R. H. Watson for kindly estimating the energy of the milk samples.

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TABLE 1.

Yield and Composition of the Milk of Six Merino Ewes on a Pasture at Adelaide, South Australia, 1933.

Period of Lactation.	*Third Week.*							*Ninth Week.*						
Sheep.	97	71	0819	W_3–B77	W_2–30	W_3–A42	Average	97	71	0819	W_3–B77	W_2–30	W_3–A42	Average
Age.	6 yr.	6 yr.	6 yr.	2 yr.	5 yr.	3 yr.		6 yr.	6 yr.	6 yr.	2 yr.	5 yr.	3 yr.	
Daily yield (gm.)	1,345	1,220	1,135	1,140	1,055	1,335	*1,205*	760	615	660	550	605	680	*650*
Specific Gravity	1·037	1·036	1·036	1·036	1·033	1·035	*1·036*	1·040	1·041	1·038	1·039	1·036	1·038	*1·039*
	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Water	83·71	82·06	80·53	82·52	83·07	82·13	*82·34*	81·93	80·72	79·58	80·00	80·03	81·98	*80·71*
Fat	5·93	7·36	8·91	7·57	6·70	8·01	*7·41*	6·46	7·48	8·29	9·34	8·97	6·87	*7·90*
Lactose	5·00	4·84	4·83	4·88	4·69	4·72	*4·83*	5·00	4·67	4·87	4·77	4·71	4·83	*4·81*
Ash	0·84	0·86	0·82	0·82	0·91	0·91	*0·86*	0·87	0·94	0·86	0·88	0·91	0·91	*0·90*
Casein (N x 6·38)	3·47	3·79	3·56	2·93	3·42	3·42	*3·43*	4·22	4·81	4·96	3·92	4·28	4·07	*4·38*
Lactalbumin	0·83	0·85	0·96	0·68	0·82	0·71	*0·81*	0·81	0·83	0·98	0·77	0·86	0·88	*0·85*
Non-protein N.	0·047	0·037	0·072	0·056	0·049	0·046	*0·051*	0·039	0·041	0·050	0·052	0·046	0·045	*0·046*
P.	0·098	0·098	0·095	0·095	0·094	0·090	*0·095*	0·12	0·13	0·14	0·13	0·12	0·13	*0·13*
Ca	0·19	0·19	0·18	0·18	0·21	0·22	*0·20*	0·21	0·20	0·20	0·20	0·22	0·20	*0·21*
Energy Calories per gm. milk	0·997	1·14	1·28	1·14	1·05	1·15	*1·13*	1·09	1·19	1·30	1·31	1·29	1·11	*1·22*

THE CHEMICAL COMPOSITION OF SOME PLANTS USED BY AUSTRALIAN ABORIGINES AS FOOD

by

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(Received for publication 10th January, 1934.)

Very little information is available regarding the composition of the foods eaten by the aborigines of Australia. For this investigation certain vegetable foods were collected in the neighbourhood of Mount Liebig, which is situated almost on the Tropic of Capricorn and 200 miles by track west of Alice Springs.[1] This area of Australia is characterized by its low rainfall and heavy alkaline soils, very different from the populated coastal regions.

An account of the methods of preparation of these various native foods has been given by Cleland and Johnston (1933). Each specimen was identified, and a quantity of the fresh substance was weighed, then partially dried and packed for transport to the laboratory. On arrival there the foods were further dried to contain approximately 10 p.c. of moisture, and the loss in weight of each food was determined. Two of the samples, namely, the leafy parts of *Portulaca oleracea* and *Calandrinia balonnensis*, were very difficult to dry, and because of this property are valued by the natives, as well as grazing cattle, as a source of moisture during the dry spells.

METHODS.

The air-dried samples were ground to pass a 30-mesh sieve. Moisture, crude fibre, ether-soluble material, total nitrogen, albuminoid and amido nitrogen, ash, sand, and silica were determined according to the methods of the Association of Official Agricultural Chemists (1930). Carbohydrate was estimated by difference.

Calcium and magnesium were estimated by the method of McCrudden (1909-10).

Potassium was determined by the cobaltinitrite method of Milne (1929).

Phosphorus was estimated by digestion of the sample with sulphuric and nitric acids, precipitation as ammonium-phospho-molybdate, solution of the precipitate in N/10 sodium hydroxide, and titration of the excess with N/10 hydrochloric acid.

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[1] The samples for this work were collected by Professors T. Harvey Johnston and J. B. Cleland in Central Australia in August, 1932, on the expedition organized by the Board for Anthropological Research of the University of Adelaide in conjunction with the South Australian Museum. This expedition was financed chiefly by a grant from the Rockefeller Foundation, administered through the Australian National Research Council. The chemical analyses were carried out in the Biochemical Laboratory of the University of Melbourne during the tenure of a Bartlett Research Scholarship for the year 1933-34.

Copper was determined by the method of Elvehjem and Lindow (1929), manganese by the method used by Richards (1930), and iron by Stugart's (1931) method.

The fuel value of each food was calculated on the basis of its composition, allowing 4·1 calories per gm. for protein and carbohydrate and 9·3 calories per gm. for fat.

DISCUSSION.

For purposes of comparison common cultivated foods were selected which are similar to the native foods studied, and analyses of both groups are included in Tables 1 and 2.

The moisture content of the fresh native foods was lower than that of similar cultivated foods. This is not surprising, as the native plants grow in an area having an annual rainfall of only 5-10 inches. Because of the wide difference in moisture content between the two classes of foods, the results were calculated on both dry and fresh basis.

The appearance of the native foods leads one to expect that their crude fibre content is greater than that of the cultivated foods. By analysis this was shown to be true of the *Solanum* fruit, the *Lepidium*, and *Portulaca* leaves, stems, and seeds, but the other native foods contained crude fibre in amounts equal to less than corresponding cultivated foods, when compared on the dry basis.

Ether-soluble material was in some cases less in the native foods.

Crude protein, which was calculated as total nitrogen x 6·25, was present in slightly greater amounts in the *Solanum* fruit and in the roots, but in lesser amounts in the other foods.

Carbohydrate, obtained by difference, was low in the *Solanum* fruit and the *Portulaca* roots.

Total ash was higher in the native foods. Sand and silica make up an appreciable amount of the total ash, although the presence of sand was not apparent when the samples were being prepared. It is very probable that in the ordinary collection and preparation of these foods by the natives the amount of sand and other inorganic constituents from the soil is even higher than represented by these analyses.

Calcium and magnesium were in most cases present in far greater amounts in the native foods. Potassium was particularly low in the *Ipomoea* tuber examined. Phosphorus was in every case lower in amount in the native foods, ranging from one-tenth to one-half of the amount in similar cultivated foods. The relation between the calcium and phosphorus is shown to be markedly different in the foods from the two sources. This is perhaps the most outstanding difference between them.

Iron was much more plentiful in the native foods. Contamination with soil particles may partially account for such a marked difference, although the copper and manganese values were not unlike those for cultivated foods.

When calculated on a dry weight basis the fuel value of the native vegetables was similar to that of the cultivated vegetables. In terms of the fresh material,

however, the fuel value of the native specimens was far greater. During and just after the rainy season such native foods are probably more succulent, and in fuel value will approach more nearly to the cultivated foods.

If the native of the Mount Liebig area subsists largely on vegetable foods of the type analysed, he may be expected to tend toward a low phosphorus intake, as six out of ten foods yielded only 0·039 to 0·394 gm. of phosphorus per 1,000 calorie portion. Corresponding cultivated foods contain 0·37 to 2·285 gm. of phosphorus per 1,000 calories. Sherman (1930a) states that 1·32 gm. of phosphorus per day and 0·68 gm. of calcium are required for adequate nutrition of an adult. Forbes (1914) favours 1·5 to 1·75 gm. of phosphorus, and Albu and Neuberg (1906) 0·7 to 1·1 gm. of calcium per day. In actual practice the natives probably receive adequate phosphorus for their needs when animal foods are available.

The low phosphorus and high calcium vegetable diet is in marked contrast with that which Cleland and Fry (1930) found was being supplied to the natives at the Hermannsburg Mission Station on the Finke River. Such a diet, consisting mainly of flour, would yield approximately 0·114 gm. of calcium and 0·597 gm. of phosphorus per day (calculated on basis of values given by Sherman (1930b). Food from the Mission was supplemented to a small extent by animals caught by the natives and a few lily buds.

Further analyses of foods eaten by natives in different parts of Australia are contemplated.

SUMMARY.

1. The composition of some vegetable foods eaten by the natives of Central Australia has been determined.

2. These native foods do not differ greatly from corresponding cultivated foods in their organic composition.

3. The inorganic constituents, with the exception of phosphorus, are plentifully supplied in the native foods.

4. Phosphorus exists in such quantities as to make it difficult for the natives to obtain adequate supplies of it if they were to live on a vegetable diet.

ACKNOWLEDGMENTS.

Acknowledgment is gratefully made to the University of Melbourne for the Research Grant whereby the analyses were made possible; to Dr. W. J. Young for his interest in the work; and to Professor F. Wood Jones, who secured the contact between the expedition which collected the samples and the Biochemistry Laboratory at the University of Melbourne.

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TABLE 1.

Composition of Native and Cultivated Foods.

Name of plant.	Part of plant used.	Moisture content.	Percentage Composition. Crude fibre. Dry Basis.	Crude fibre. Fresh Basis.	Ether extract. Dry Basis.	Ether extract. Fresh Basis.	Crude protein. Dry Basis.	Crude protein. Fresh Basis.	Ash. Dry Basis.	Ash. Fresh Basis.	Carbohydrate(f) Dry Basis.	Carbohydrate(f) Fresh Basis.	Albuminoid Nitrogen. Dry Basis.	Albuminoid Nitrogen. Fresh Basis.	Amido Nitrogen. Dry Basis.	Amido Nitrogen. Fresh Basis.	Fuel Value(e) Dry Basis.	Fuel Value(e) Fresh Basis.
Solanum ellipticum, R.Br., Solanaceae	Fruit	86	18·9	2·65	12·6	1·77	18·8	2·63	8·92	1·25	40·7	5·70	2·62	0·36	0·39	0·05	27·6	197
Tomato(c)	„	91·9	13·0	1·10	2·50	0·20	16·0	1·30	8·70	0·70	62	5·0	—	—	—	—	29·1	361
Boerhavia diffusa, L., Nyctaginaceae	Root	50	9·6	4·80	1·62	0·81	9·1	4·56	8·42	4·21	71·2	35·6	1·02	0·51	0·44	0·22	29·0	58
Vigna Lanceolata, Benth., Leguminosae	„	76	6·6	1·57	1·32	0·31	11·8	2·82	6·71	1·59	73·5	17·5	1·48	0·35	0·42	0·09	27·6	116
Portulaca oleracea, L., Portulacaceae	„	79	9·2	1·94	1·83	0·38	16·8	3·54	12·78	2·68	40·7	8·5	2·07	0·43	0·63	0·13	39·5	188
Carrot (b)	„	86·5	10·5	1·50	2·1	0·3	3·5	0·5	6·3	0·9	70·6	10·1	—	—	—	—	30·9	216
Parsnip (a)	„	80·1	10·5	2·10	5·0	1·0	7·0	1·4	6·5	1·3	70·5	14·1	—	—	—	—	27·4	*137.*
Turnip (b)	„	90·3	18·0	1·80	1·5	0·15	9·0	0·9	8·0	0·8	50·0	5·0	—	—	—	—	39·0	403
Ipomoea sp. (g), Convolvulaceae	Tuber	40(d)	6·9	4·15	1·30	0·78	3·3	1·98	5·14	3·08	86·5	51·9	0·41	0·24	0·12	0·07	26·2	43·8
Sweet Potato (a)	„	72·9	6·6	1·8	1·8	0·5	5·9	1·6	2·5	0·7	83·3	22·5	—	—	—	—	26·1	96·6
Lepidium papillosum, F. v. M., Cruciferae	Leaves, small stems.	51·5	20·0	9·7	2·29	1·11	17·8	8·63	8·15	3·95	51·7	25·1	1·91	0·92	0·93	0·45	32·6	67·3
Marsdenia australis, (R.Br.), Black, Asclepiadaceae	Leaves, small green twigs.	73	6·42	1·68	6·97	1·88	20·4	5·52	11·90	3·21	54·3	14·6	2·93	0·79	0·34	0·09	26·9	100
Portulaca oleracea L., Portulacaceae	Leaves, stems, seeds.	89	13·8	1·52	3·43	0·37	19·1	2·09	18·70	2·06	45·0	4·9	2·01	0·22	1·04	0·11	33·9	309
Celendrinia balonnensis, Lindl.,	Leaves	93	9·7	0·68	2·19	0·15	16·8	1·17	21·38	1·49	49·9	3·5	1·63	0·11	1·06	0·07	34·0	487
Cabbage (c)	„	89·6	11·1	1·1	4·0	0·4	11	1·1	13·0	1·3	58	5·8	—	—	—	—	30·9	309
Lettuce (c)	„	94·1	8·3	0·5	6·6	0·4	23	1·4	16·0	1·0	43	2·6	—	—	—	—	30·1	502
Spinach (c)	„	90·6	9·0	0·9	5·0	0·5	25	2·5	17·0	1·7	38	3·8	—	—	—	—	32·8	329
Marsdenia australis, (R.Br.), Black, Asclepiadaceae	Fruit	77·7	50·2	11·2	9·14	2·04	15·2	3·38	3·79	0·84	21·6	4·8	1·90	0·42	0·53	0·12	42·4	190

(a) Hutchinson and Mottram (1933a); (b) Hutchinson and Mottram (1933b); (c) Hutchinson and Mottram (1933c); (d) Partly dry; (e) Grams per 100 calories; (f) Carbohydrate by difference; (g) Probably I. *calobra*, Hill *cf.* F.v.M.

TABLE 2.

Inorganic Composition of Native and Cultivated Foods.

Name of plant.	Part of plant used.	Percentage Composition. Sand. Dry Basis.	Sand. Fresh Basis.	Silica. Dry Basis.	Silica. Fresh Basis.	Calcium. Dry Basis.	Calcium. Fresh Basis.	Magnesium. Dry Basis.	Magnesium. Fresh Basis.	Phosphorus. Dry Basis.	Phosphorus. Fresh Basis.	Potassium. Dry Basis.	Potassium. Fresh Basis.	Ca / P	Fe(e) in p.p.m. on dry basis	Cu(d)	Mn(e)
Solanum ellipticum, R.Br., Solanaceae	Fruit	0·45	0·06	0·17	0·02	0·250	0·035	0·260	0·036	0·145	0·020	2·54	0·355	1·72	357	12	26
Tomato (a)	„	—	—	—	—	0·125	0·011	0·122	0·010	0·325	0·026	3·43	0·275	0·42	103	10	—
Boerhavia diffusa, L., Nyctaginaceae	Root	0·34	0·17	0·22	0·11	1·246	0·623	0·344	0·172	0·155	0·077	2·89	1·440	8·03	345	6	20
Vigna Lanceolata, Benth., Leguminosae	„	0·63(b)	0·15(b)	—	—	0·745	0·177	0·53	0·127	0·157	0·037	3·25	0·774	4·74	449	4	17
Portulaca oleracea, L., Portulacaceae	„	0·89	0·18	0·48	0·10	1·068	0·224	0·289	0·061	0·279	0·058	5·60	1·170	3·82	830	9	37
Carrot (a)	„	—	—	—	—	0·390	0·056	0·140	0·021	0·320	0·046	2·00	0·287	1·21	108	8	6
Parsnip (a)	„	—	—	—	—	0·295	0·059	0·170	0·034	0·480	0·076	2·59	0·518	0·77	62	7	13·9
Turnip (a)	„	—	—	—	—	0·640	0·064	0·170	0·017	0·460	0·046	3·38	0·338	1·39	82	11	—
Ipomoea sp. (f) Convolvulaceae	Tuber	0·270	0·162	0·10	0·06	0·743	0·446	0·116	0·064	0·016	0·009	0·208	0·125	46·3	238	6	17
						0·070	0·019	0·010	0·028	0·166	0·045	1·470	0·397				5·4
Sweet Potato (a)	„	—	—	—	—									0·42	33	5·2	19·6
Lepidium papillosum, F. v. M., Cruciferae	Leaves, small stems.	0·200	0·097	0·25	0·12	0·940	0·455	0·417	0·202	0·059	0·029	3·433	1·665	15·7	210	6	17
Marsdenia australis, (R.Br.), Black, Asclepiadaceae	Leaves, small green twigs.	0·130	0·035	0·19	0·05	2·26	0·610	1·001	0·270	0·210	0·057	3·240	0·875	10·7	191	4	137
Portulaca oleracea L., Portulacaceae	Leaves, stems, seeds.	1·88(b)	0·207(b)	—	—	1·022	0·112	1·872	0·206	0·218	0·024	6·450	0·709	4·68	1029	17	21
Calandrinia balonnensis, Lindl., Portulacaceae	Leaves	0·99(b)	0·069(b)	—	—	0·301	0·021	1·064	0·074	0·055	0·004	11·400	0·798	5·47	268	11	32
Cabbage (a)	„	—	—	—	—	0·450	0·045	0·150	0·015	0·290	0·029	2·47	0·247	1·55	46	6·8	10·6
Lettuce (a)	„	—	—	—	—	0·710	0·043	0·280	0·017	0·700	0·042	5·60	0·339	1·02	330	11·6	216
Spinach (a)	„	—	—	—	—	0·670	0·067	0·370	0·037	0·680	0·068	7·74	0·774	0·98	814	6·9	86·5
Marsdenia australis, (R.Br.), Black, Asclepiadaceae	Fruit	0·27	0·06	—	—	0·100	0·022	0·203	0·045	0·166	0·037	—	—	0·60	120	—	19

(a) Inorganic composition of cultivated foods, Sherman (1930b); (b) Sand and Silica; (c) Peterson and Elvehjem (1928); (d) Lindow, Elvehjem and Peterson (1929); (e) Lindow and Peterson (1927); (f) Probably I. *calobra*, Hill *cf.* F.v.M.

ALTERATIONS IN THE CALCIUM CONTENT OF THE BLOOD IN RELATION TO THE MENSTRUAL CYCLE

by

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(Submitted for publication 14th February, 1934,)

In 1929 one of us, Matters (1929), published the results of investigations on the calcium content of the blood during the menstrual cycle of the human female. These investigations had been made on a number of females over a period of two years. The results indicated a premenstrual rise in the calcium content of the blood, followed by a fall which began immediately before the onset of menstruation.

This was a preliminary investigation, and as the regular analysis of the blood was not satisfactory, it was decided that further work, in order to confirm these findings, should be undertaken.

A series of twelve women was used in this investigation over a period of five months. All of these subjects had a normal menstrual rhythm and flow. The blood was obtained at weekly intervals from eleven of these subjects; and from one (E.U.H.), who volunteered a daily specimen for analysis, blood samples were taken each day over a period of three months. The estimations of the calcium were carried out in triplicate by one of us (E.U.H.) according to the Clark-Collip (1925) modification of the method of Kramer and Tisdall (1921).

It was thought that the variations obtained in the preliminary study were due to alterations in endocrine secretion, especially as Mirvish and Bosman (1927) had produced variations in the calcium content of the blood of rabbits by the injection of ovarian hormones.

Further, one of us (R.F.M.) found that rhythmical changes in the gaseous metabolism occurred during the menstrual cycle, indicating rhythmical variations in the thyroid activity. We considered that the differences in the amounts of calcium in the blood were due to action of the hormone of the parathyroid glands, the activity of which, it was considered, varied with the menstrual cycle.

The curve in Chart I (E.U.H.) shows the results of the estimations of the calcium of the blood taken daily at the same hour from November 28 until Feb-

ruary 10 inclusive. It will be seen that there is no regular variation in the calcium content in relation to menstruation, which occurred on December 14, 15, 16, 17, on January 10, 11, 12, 13, and on February 4, 5, 6, 7. This record, taken daily over a period of three menstrual cycles, seemed to offer the most accurate information.

There does, however, appear to be some increase of the blood calcium content at what is regarded as being the time of rupture of the Graafian follicle. We found that during pregnancy, when the folliculin in the blood-stream increases, so also does the blood calcium content. We have also found that the blood serum of women at the time of rupture of the Graafian follicle, when injected into female mice produced a positive reaction for folliculin, and this was demonstrated both by the vaginal smear and by Sidall's method of recording the engorgement of the uterus and adnexa.

It is manifest that there is some increase of blood calcium on November 2, December 29, and January 24, and, if it might be assumed that this is the time of dehiscence of the follicle and the sudden outpouring of folliculin, it might be regarded as indicative of some parathyroid response to the follicular hormone.

Charts II, III, IV, V, and VI show the results obtained from weekly estimations of the calcium content of the blood from five out of the series, over a period of five months.

CONCLUSIONS.

The alterations in the calcium contents of the blood during the menstrual cycle, reported by one of us (R.F.M.) in a previous paper have not been confirmed. We can find no relationship between changes in the calcium content of the blood and the menstrual cycle.

There is an indication of cyclical variations which possibly bear some relationship to the ovarian cycle. i.e. the dehiscence of the Graafian follicle and the increase of folliculin in the blood stream.

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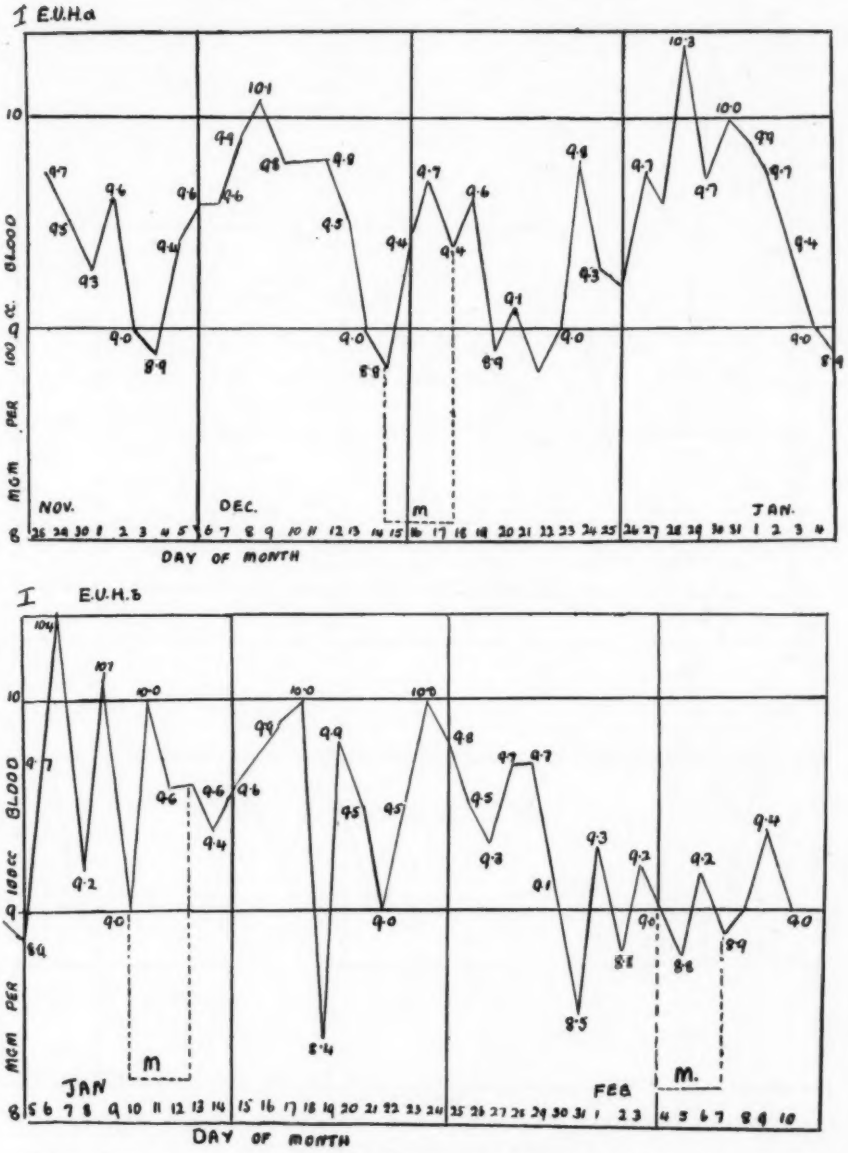
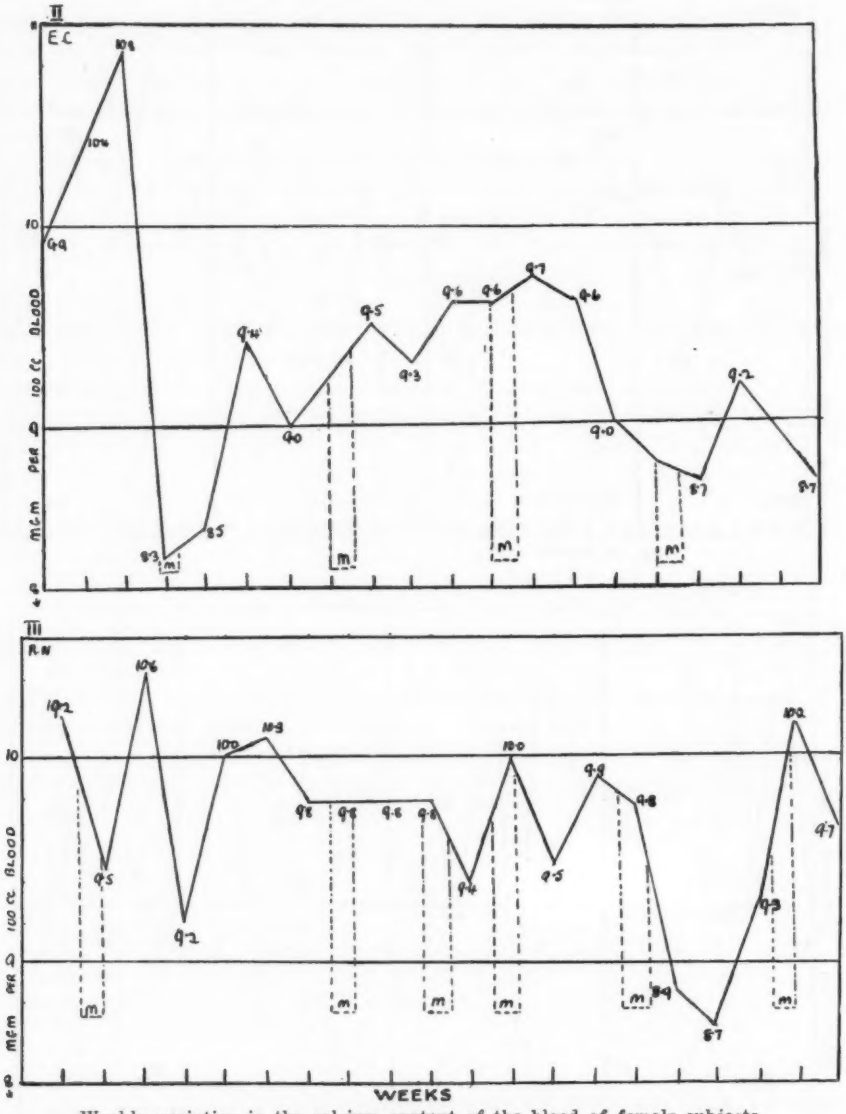
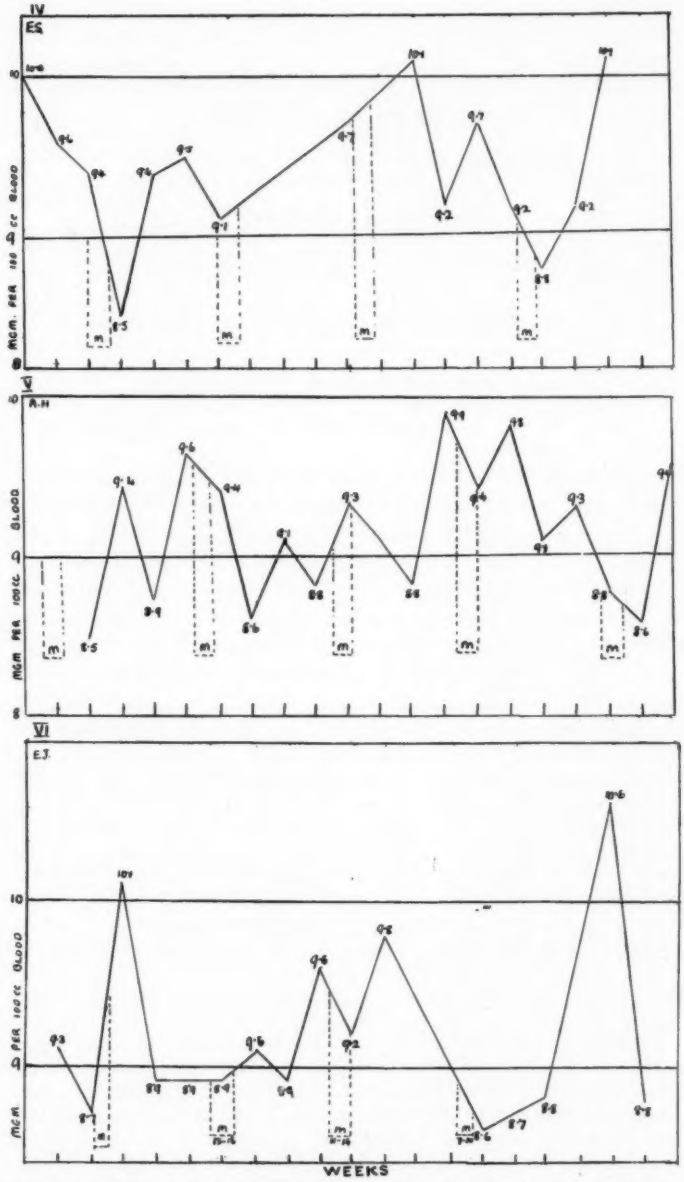


Daily variation in the calcium content of the blood of a female subject for three months. M denotes the periods of menstrual flow.



Weekly variation in the calcium content of the blood of female subjects.
M denotes the periods of menstrual flow.



Weekly variation in the calcium content of the blood of female subjects. M denotes the periods of menstrual flow.

NEW TREMATODES FROM SOUTH AUSTRALIAN ELASMOBRANCHS

by

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(Zoology Department, University of Adelaide).

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CALICOTYLE AUSTRALIS n. sp.

(Figs 1-3.)

A solitary individual belonging to the genus *Calicotyle*, now recorded for the first time as occurring in Australia, was found on a fiddler ray, *Trygonorrhina fasciata* Muller & Henle (Rhinobatidae), caught off Glenelg, South Australia. Its general anatomy resembles that of *C. kroyeri* Dies. from European rays (*Raia* spp.) and *C. mitsukurii* Goto from a Japanese species of *Rhina*. Excellent accounts of these two parasites have been published by Wierzejski (1877) and Goto (1894) respectively. Scott's figure of *C. kroyeri* resembles *C. australis*, but practically no anatomical details are given (1902).

The body of *C. australis* is rather elongate, widened posteriorly, with a length of about 6·2 mm. from the anterior end to the base of the cotylophore, but, if this be included, the worm measures 7·2 mm. The maximum breadth is 3·5 mm. The ratio of total length to breadth is thus about 2 : 1. In *C. kroyeri* it is approximately 6 : 5, and in the Japanese form about 8 : 5. The large sessile posterior disc is about 1·7 mm. in diameter with its eight loculi typically arranged, the median posterior loculus being the largest. The disc projects beyond the body to almost half the length of the former. The two hooks are rather stout with the points strongly incurved, their form being more like those of *C. kroyeri*, as figured by Goto. The length, not including the curved tip, is about 0·34 mm., that of *G. kroyeri* being about 0·30 mm. (according to Goto), and of *C. mitsukurii* 0·56 mm.

At the anterior end are the four cephalic glands, the ducts having rather thick walls. The inner portions could not be traced with certainty, but they appear to be very much shorter than those described by Goto and they do not extend beyond the level of the mouth. The latter is a short distance from the anterior end, transversely placed, and surrounded by the prominent anterior sucker whose structure is similar to that described by Wierzejski and by Goto. There is no basement membrane, and the fibres pass out to be inserted into various regions, many of them becoming longitudinal. The circular fibres are very abundant and delicate.

The strongly muscular pharynx is succeeded by a very short oesophagus. As in the other two species, there is a slight incurving of the intestinal crura, just in front of the level of the ovary, while the terminal portion curves inwards towards its fellow and then somewhat posteriorly, but rather more so in *C. australis* than in the other species. The region between the

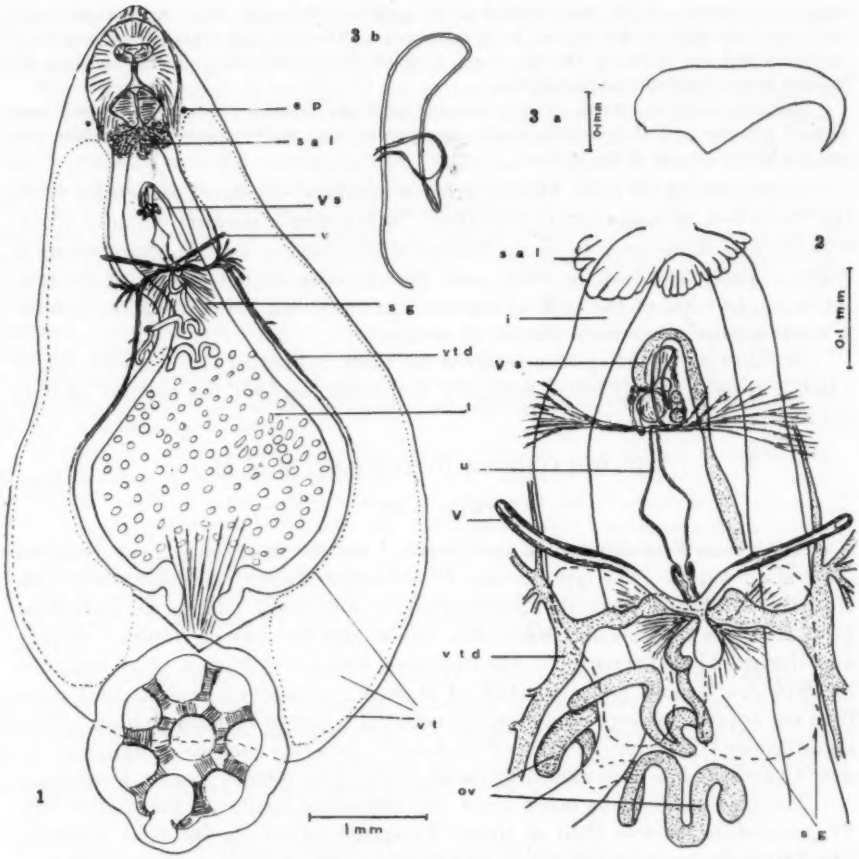
ends of the cura is traversed by numerous powerful longitudinal muscular fibres which enter the disc. Unicellular salivary glands are abundant, occupying the region in the vicinity of the junction of the crura and oesophagus, where they occur laterally and posteriorly, lying above the commencement of the crura.

The small, irregularly-shaped, testes are very numerous, and fill the region between the crura. They extend posteriorly from the ovary to the region where the crura approximate. Some vesicles underlie the intestine. Their appearance is more like those of *C. mitsukurii*. The vas deferens passes forwards on the left side, just inwardly from the corresponding crus. It then becomes more or less swollen and migrates towards the midline, a little distance behind the intestinal bifurcation. It now bends backwardly on the right side of the median line as a much swollen vesicula seminalis with whose posterior end is associated the ejaculatory apparatus. The male canal, after receiving the ducts of these accessory structures now becomes well chitinized and funnel-shaped, continuing as a very narrow chitinous penis, 0·4 mm. long (measuring along the curves). It is thus shorter than that of *C. mitsukurii* (0·59 mm.) which it otherwise closely resembles. Its first part travels backwards to become bent sharply on itself dorsally, then forwards in a curved course to lie below the vesicula, and then backwards below the vesicula and ejaculatory apparatus, almost in a straight line to the genital pore. The vesicula is the most dorsally situated portion of the male duct. The ejaculatory apparatus differs from those described for the other two species, in which the structure is fairly similar. An elongate diverticulum of the vesicula occurs on the right in *C. australis*. Instead of the two prominent ejaculatory bulbs, only one, lying medianly, was observed, but there are three smaller, deeply staining sacs, perhaps prostate glands, associated with the apparatus. Two of these sacs lie in the position occupied by an ejaculatory bulb in the other species and may perhaps represent that organ, but the staining reactions are quite different.

The ovary is similar in form to that of the other species, lying transversely just in front of the testicular field and thrown into several folds or loose coils as it travels to the right, passing above the corresponding crus and around it to recross it ventrally and a little more anteriorly. It is then thrown into a few irregular curves near the midline and in front of the rest of the ovary, eventually travelling forwards just above the transverse yolk duct, to enter the fertilizing duct immediately in front of the termination of the yolk duct. The ootyp then passes forwards as a short tube with wide walls of rather loose tissue, within which the uterine duct may have a wavy course. Into its posterior portion there enter the two groups of ducts belonging to the shell gland. The canal suddenly changes into a broader, somewhat spindle-shaped, uterus (ootyp of Goto) which terminates in a short narrow tubular part (uterus of Goto) ending at the genital pore adjacent to, and immediately behind, the male aperture. The uterus is about 0·15 mm. long from the end of the ootyp to the female pore. An egg was not present in the specimen examined.

The paired vaginae open ventrally at about the level of the mid-uterus, just laterally from the intestinal crura. The outer portion of each has considerably thickened glandular walls, as indicated by Goto and by Wierzejski. These ducts travel inwards and slightly posteriorly to join in the midline below the transverse yolk duct, and then communicate with the rather elliptical receptaculum seminis, which lies in the midline alongside the terminal portion of the oviduct. The receptaculum connects anteriorly and dorsally with the oviduct near the vaginal entrance. After receiving the yolk duct ventrally, the oviduct passes forwards, as the ootyp, above the united vaginae and the yolk duct.

The shell glands occupy an extensive region between the ovary and lateral vaginae and between the intestinal crura, some of the cells lying below the crura and the vas deferens. The ducts are very long and, as already stated, enter the commencement of the ootyp in two groups, a right and a left.



Figs. 1–3. *Calicotyle australis.* 1. ventral view; 2. reproductive system; 3a. hook from cotylophore; 3b. penis.

Reference to lettering: e.p. excretory pore; i. intestine; od. oviduct; ov. ovary; r.s. receptaculum seminis; sal. salivary glands; s.g. shell glands; t. testes; t.vt.d. transverse vitelline duct; u. uterus; v. vagina; v.s. vesicula seminalis; vt. vitellarium; vt.d. vitelline duct.

The vitellaria lie outwardly from the intestinal crura and extend from the level of the middle of the pharynx to the posterior end of the body. The arrangement is thus similar to that in other species. The ducts unite to form a transverse canal which lies between the receptaculum and the vaginae, and on a more dorsal level than these. This canal gives off a very short common duct which passes upwards to enter the female duct in the vicinity of the junction of the oviduct and the receptaculum.

Connected with the genital atrium is a peculiar organ consisting of a right and left series of delicate branching tube-like structures ramifying in the ventral tissues and extending laterally

below the intestine and the inner portion of the adjacent vitellaria. Their contents are very finely granular and are not stained by haematoxylin. They lie just behind the ejaculatory apparatus and are probably the structures seen by Wierzejski, who referred to them as bundles of fine fibres resembling nerves.

The main excretory canals lie just laterally from the intestinal crura. The pore opens dorsally near the level of the middle of the pharynx and about midway between the median line and the lateral margin of the worm.

Calicotyle affinis Scott (1911), from the gills of *Chimaera monstrosa* from the North Sea, is inadequately described. It is a larger parasite, broadly ovate, with its maximum breadth in the posterior two-thirds. Scott's figure shows a different distribution of the testes, and the vitellaria do not extend so far posteriorly. In view of the lack of information regarding the rest of the genital system, further comparison cannot be made.

In addition to the species referred to, there is another, *C. stossichi* Braun (1899), from *Mustelus laevis* from the Mediterranean, but the account of it is not available.

PROBOLITREMA ROTUNDATUM n. sp.

(Figs. 4-5.)

The genus *Probolitrema* is now recorded for the first time as occurring in Australian waters. The type species, *P. richiardii* (Lopez), was described from *Acanthias vulgaris* from the Mediterranean, Monticelli adding as additional hosts *Mustelus vulgaris* and *Myliobatis aquila* from the Bay of Naples. Shipley and Hornell (1905) reported it (as *Distomum richiardii*) from a shark regarded as *Rhinodon typicus* from the Gulf of Manaar. It is quite possible that more than one species was represented in the material collected from these hosts. One, identified by Ofenheim (1900) as *P. richiardii*, from the coelome of *Scyllium* sp. near Capetown, has been named by Looss (1902) as a distant species, *P. capense*.

The new species was taken from the abdominal cavity of the fiddler ray, *Trygonorrhina fasciata* Mull. & Henle, Kangaroo Island, by Dr. C. J. Hackett, who found specimens in one out of six examined. The largest measures 6 mm. in length by 5 mm. in breadth. The anterior sucker has a diameter of 0·8 mm., and the posterior 1·2 mm. (uncompressed), the ratio being 2 : 3.

There is a short pharynx about 0·4 mm. long by 0·6 mm. wide, largely covered by the anterior sucker. The oesophagus is comparatively wide. The intestinal crura are also wide and are thrown into a few curves, while the walls show the presence of numerous small saeculations on both sides, especially posteriorly.

There are about 25–28 testes on each side, lying laterally from the crura from a point a little behind the vitellaria, and extending to a point some distance in front of the termination of the crura. In one specimen these glands are seen to extend forwards to overlie part of the yolk gland; in another a few vesicles underlie the crus, and two are actually inwardly from the crus and partly above the uterine tube. The individual glands vary considerably in form and size, and all of them show distinct irregular lobulation. They are arranged in two more or less defined layers. The vesicula seminalis is a small rounded organ, about 0·2 mm. in diameter,

lying in the midline just in front of the ventral sucker and a short distance behind the genital pore, which is situated immediately below or behind the intestinal bifurcation. There is no cirrus, the vesicula connecting with the pore by a male duct.

The ovary lies slightly to the right of the midline and is distinctly trilobed. Though the organ is compact in young specimens just entering on egg-bearing, it is somewhat elongate transversely in older worms, the three lobes becoming rather pedunculate. Each lobe has a few short lobules, some of them projecting ventrally. The oviduct is very short, travels forwards and inwards towards the midline, receiving the narrow curved duct from the large receptaculum seminis. The latter has a diameter of about one millimetre, and lies on the right in front of the ovary and partly overlies the ventral sucker. The elongate inconspicuous shell gland is nearly median, and lies immediately in front of the level of the ovary and in front of the transverse yolk duct. The first part of the uterus has a sinuous course behind the receptaculum and in front of the transverse vitelline duct, but after crossing the latter it becomes thrown into very numerous convolutions which occupy practically the whole region between the crura. Part of the organ extends posteriorly to the ends of the crura, while anteriorly it borders on the ovary and receptaculum, and overlies part of the acetabulum, becoming narrowed to travel to the left of the vesicula and terminate alongside the male duct. Eggs measure ·050 to ·054 mm. in length by ·036 to ·039 mm. in breadth.

The vitellaria form a compact shrub-like organ on each side, about 0·7 mm. in length, lying at about the level of the posterior border of the acetabulum and partly underlying the crura. Each gland consists of a few closely arranged branches composed of a number of very short lobes. The duct passes inwards and somewhat backwards below the intestine and uterus to meet its fellow in front of the ovary, where it forms a very short common duct which joins the oviduct just before the latter enters the shell gland.

The excretory pore is terminal, leading into a fairly extensive excretory vesicle lying between the ends of the crura and below the uterine tube. Its further course inwards was not traced.

As Ofenheim's account, on which Looss' name is based, is not available, a comparison with *P. capense* cannot be made, but *P. rotundatum* differs from the type species in the form, body proportions, sucker ratio, form of vitellaria, and form of excretory vesicle, while the characters of the testes and crura readily distinguish it from the two species described below.

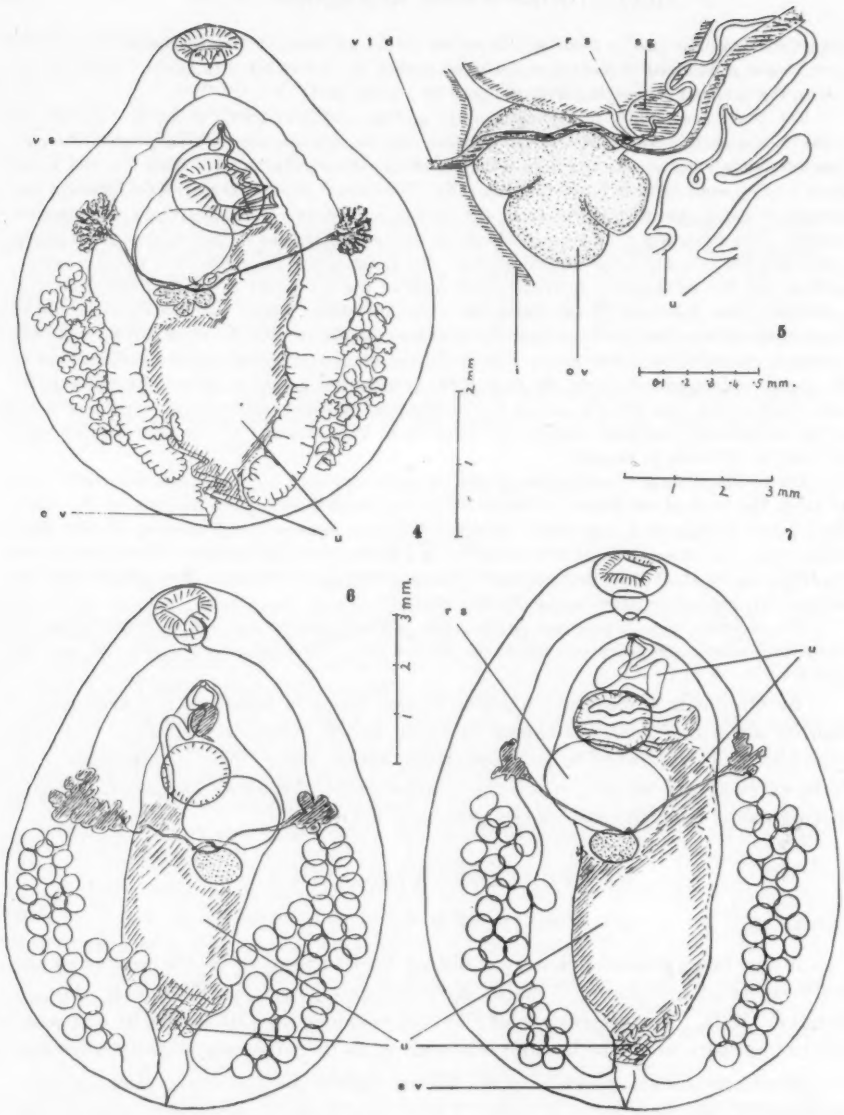
PROBOLITREMA CLELANDI n.sp.

(Fig. 6.)

A few large trematodes were collected by Professor J. B. Cleland from the body cavity of a "gummy" shark, *Mustelus antarcticus*, at Encounter Bay, in January, 1922. The dimensions of the type specimen are 10·5 mm. by 7·7 mm., but the parasite would be broader when alive, as the edges are slightly inturned.

The surface is minutely scaly or spiny. The anterior and posterior suckers exhibit punctate surfaces, and have diameters of 1·2 and 1·3 mm. respectively, the acetabulum being flat and comparatively weak. The pharynx measures 0·5 mm. long and 0·7 mm. broad. The oesophagus is short and the crura wide, and devoid of obvious sacculations.

The type possesses 31 rounded testes on one side and 37 on the other. They are not restricted to the region laterally from the crura, but extend inwardly to underlie them, and a few vesicles are present in the intercrural area also. They extend from the posterior border of the



Figs. 4–5. *Probolitrema rotundatum.* 4. ventral view; 5. ovary, etc., of young specimen, just entered on egg-bearing.

Fig. 6. *Probolitrema clelandi.*

Fig. 7. *Probolitrema simile.*

Reference to lettering: e.p. excretory pore; i. intestine; od. oviduct; ov. ovary; r.s. receptaculum seminis; sal. salivary glands; s.g. shell glands; t. testes; t.vt.d. transverse vitelline duct; u. uterus; v. vagina; v.s. vesicula seminalis; vt. vitellarium; vt.d. vitelline duct.

vitellaria, almost to the end of the crura. They measure 0·3 to 0·5 mm. in diameter. The vasa deferentia unite in the region of the ventral sucker to form a small vesicula seminalis about 0·6 mm. long by 0·35 mm. wide, which is succeeded by a male duct leading to the genital aperture lying under the intestinal bifurcation and about a millimetre in front of the anterior edge of the ventral sucker.

The ovary is compact, nearly median, one millimetre across and 0·65 mm. in length, and situated about a millimetre behind the posterior border of the acetabulum. The oviduct is very short and leads forwards to the small shell gland which lies adjacent to the ovary, between it and the large receptaculum seminis. The latter is 1·4 mm. in length and 2 mm. in breadth, and partly overlies the ventral sucker. The uterus occupies the intercrural region behind the level of the ovary, but its convolutions do not extend backwards as far as the crural terminations. It passes forwards above and to one side of the sucker, becoming thrown into a few folds in front of the latter, some of them being below the vesicula. Eggs have a small projecting polar thickening, and measure ·074 to ·065 mm. in length by ·057 to ·049 mm. in width.

The compact vitellaria are situated at the level of the receptaculum and lie partly outwardly from and largely ventrally to the crura. Each has a number of short rounded lobes. The ducts pass inwards and slightly posteriorly to meet and form a very short common duct at the anterior border of the ovary where the yolk duct joins the oviduct as it enters the shell gland.

There is a wide excretory vesicle between the ends of the crura. Its forward limit was not determined.

As the receptaculum and ovary are largely to the left of the median line and the uterus passes forwards on the right of it, it is possible that the arrangement is an amphitypy.

The systematic position of this species is doubtful. The general organization suggests a *Probolitrema*, but the arrangement of the testes and vitellaria are not typical of that genus as diagnosed by Looss, Travassos, and Nagaty. As, however, testicular vesicles may appear intercrurally on one side in other species, and since the yolk glands are partly overlapped by the intestine in the species already described, it appears preferable to amend the generic diagnosis rather than erect a new genus. The following is suggested: Large Anaporrhutinae with posterior region broadened; strongly muscular pharynx; intestinal caeca not branched, though sacculations may be present; testes consisting of numerous follicles entirely or mainly extracural; vitellaria entirely or partly extracrural; parasites of the body cavity of Elasmobranchs.

PROBOLITREMA SIMILE n. sp.

(Fig. 7.)

In addition to *P. clelandi*, a few specimens of a closely related trematode were obtained from *Mustelus antarcticus* at Encounter Bay by Professor Cleland. The dimensions are: length, 11 to 13 mm.; breadth, 8 mm.

The surface is very minutely scaly or spiny. The anterior and posterior suckers have a diameter of 1·4 and 1·7 mm. respectively. They have punctate surfaces. The intestine is devoid of sacculations. There are about 32 rounded or slightly elliptical testes on each side, measuring 0·3 to 0·5 mm. in diameter. Though largely restricted to the extracural region, some vesicles partly underlie the crura. They extend from the yolk glands almost to the ends of the crura. The vesicula is similar to that of *P. clelandi*, but rather longer, being about 1 mm. in

length. The genital pore lies immediately behind the intestinal bifurcation. The compact ovary is slightly to the right of the midline, and measures about a millimetre across. The receptaculum is very large, being about 2 mm. long by 2·5 mm. in width, and with its axis somewhat to the right of the median line.

The shell gland is very small, and lies adjacent to the anterior border of the ovary, the oviduct being extremely short. The uterus soon crosses the left yolk duct and travels posteriorly, its convoluted tube occupying the intercrural region and extending a little beyond the ends of the crura. Eggs have a polar thickening and measure, in utero, 0·05 to 0·042 by 0·040 to 0·037 mm.

The obvious difference between *P. clelandi* and *P. simile* is the distribution of the testes. Perhaps an examination of a large series may show that there are intermediate forms, and that both should be united under the former name.

The types of the four new species are deposited in the South Australian Museum, Adelaide.

In addition to the species of *Probolitrema* referred to in this paper, other trematodes belonging to the Anaporrhutinae have been recorded from Australia, S. J. Johnston (1913) having described *Petalodistomum polycladum* and *P. cymatodes* from the leopard ray, *Dasybatis kuhli*, from North Queensland. The latter species was subsequently transferred to *Staphylorchis* by Travassos (1922).

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THE INFLUENCE OF INTERMITTENT STARVATION AND OF INTERMITTENT STARVATION PLUS NUCLEIC ACID ON THE GROWTH AND LONGEVITY OF THE WHITE MOUSE

by

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During the last two decades Robertson carried out many experiments in which he sought a clue that would provide a guide in the elucidation of the mysteries of the process of senescence.

The hypothesis which stimulated these researches was primarily based on the observation of Hertwig (1903), who showed that division occurs in any given species of cell when the ratio of the volume of the nucleus to the volume of the whole cell reaches a certain value.

It follows that if anything should happen to delay the growth of the nucleus in comparison with the growth of cytoplasm, so that the critical ratio would not be attained, then the division of the cells could no longer occur and stasis of growth in the cell community must result. The origin of the deterioration of animal tissues with age, and the consequent mortality of complex cell communities, might reside in such a gradual falling off of the nucleo-cytoplasmic ratio of certain tissue cells below the limits compatible with healthy function. The proportion of the nuclear materials in the cells is known to diminish throughout life —at first rapidly and later more slowly—(LeBreton and Schaeffer (1923), Dawbarn (1932)), and it seems not improbable that death, which supervenes on the impaired individual efficiency of all tissue cells, is the ultimate outcome of the process of growth.

If, then, duration of life is set by events and characteristics which are internal, and are attributable to progressive physico-chemical changes occurring in the tissues of the animal itself, it is conceivable that these changes might be modified, and if means could be found to accomplish this, then the duration of life of animals should be susceptible to control by experimental procedures.

Robertson exploited two main methods of attack in his attempts to alter the nucleo-cytoplasmic ratio of the growing animal. He tried to augment nuclear synthesis by increasing to an abnormal extent the nutriments known to be required for the elaboration of nucleic acid; he attempted, on the other hand, to diminish the growth of cytoplasm, whereby the decrease in the ratio with age would be arrested by preventing the progressive increase of the denominator of the fraction.

Feeding relatively massive doses of nucleic acid derived from thymus or from yeast resulted in some increase in the duration of life in the experimental animals, as did also ingestion of large doses of thyroid autocoid during pre-adolescence.

In the following experiments, which constitute the last of the series started by Robertson prior to his death in 1930, attempts were made to reduce the cytoplasmic mass by withdrawing food from the experimental animals two days in every seven, and hopes were entertained of supplying materials for nuclear synthesis by superimposing a daily dose of 25 mg. of yeast nucleic acid on the treatment.

Each experimental unit consisted of twenty-four mice, which were selected, cared for, and their behaviour tabulated as previously described (Robertson, 1925).

The results supervening on intermittent starvation of both males and females are contrasted with control groups of each sex, and the effect of adding 25 mg. of yeast nucleic acid to the daily ration are expressed in figs. 1-4.

During the two-day fasting periods the mice lost over 12 p.c. of their body-weight, but this was rapidly regained after they had access to food, and, while the immediate effect of intermittent starvation was to reduce the growth rate of all groups, the mean body-weight of the animals so treated sooner or later became greater than that of the controls, which continually received a superabundance of the same food. This effect was especially noticeable in the male animals. Feeding 25 mg. per day of yeast nucleic acid to the fasted animals had little further influence either on their growth or longevity.

While the periodic abstinence from food did not significantly increase the life duration, it is remarkable that such treatment certainly did not decrease the expectancy of life, and, furthermore, the significant increase in the body-weight of the male animals which followed such treatment is most striking when we consider the relatively high rate of energy consumption of the small rodent.

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TABLE 1.

Control Males.

Number of animals.	Age in weeks.	Weight in gm.	Probable error of mean. + or —	Number of animals.	Age in weeks.	Weight in gm.	Probable error of mean. + or —
23	5	3·39	·16	20	62	29·90	·45
23	6	10·00	·23	20	64	30·05	·48
23	7	11·89	·31	20	66	30·35	·47
23	8	13·50	·31	20	68	30·00	·49
23	9	15·13	·28	20	70	29·60	·42
23	10	17·13	·33	19	72	30·00	·43
23	11	18·33	·31	19	74	30·00	·47
23	12	19·35	·34	19	76	29·89	·47
23	13	20·41	·41	19	78	29·00	·42
23	14	21·00	·36	18	80	29·86	·50
23	15	21·80	·38	18	82	29·89	·59
23	16	22·50	·37	17	84	30·00	·54
23	17	23·43	·39	17	86	29·82	·53
23	18	23·78	·34	16	88	29·97	·56
23	19	23·87	·30	16	90	30·09	·50
23	20	24·43	·29	16	92	29·75	·52
23	21	25·04	·32	15	94	29·60	·54
23	22	25·65	·33	15	96	29·33	·52
23	23	26·33	·32	14	98	29·32	·41
23	24	26·22	·34	14	100	29·28	·62
23	25	26·80	·34	13	102	29·00	·86
23	26	26·85	·35	12	104	29·12	·55
23	27	27·02	·35	12	106	29·04	·60
23	28	27·63	·40	10	108	28·85	·82
23	29	27·76	·44	9	110	29·94	·56
23	30	27·28	·46	9	112	29·16	·66
23	32	26·83	·39	8	114	28·18	·88
23	34	28·13	·44	8	116	28·81	·83
23	36	28·93	·48	7	118	29·00	1·11
23	38	28·61	·41	6	120	29·75	·98
23	40	28·26	·36	6	122	29·75	1·13
22	42	28·73	·43	6	124	29·16	1·23
22	44	28·68	·42	6	126	28·58	1·06
22	46	29·27	·42	4	128	30·25	—
21	48	28·88	·51	4	130	27·75	—
21	50	28·74	·45	2	132	28·25	—
21	52	28·67	·49	2	134	25·75	—
21	54	29·14	·46	2	136	27·00	—
21	56	29·62	·47	2	138	26·50	—
21	58	28·71	·40	2	140	24·50	—
20	60	29·12	·42	2	142	24·25	—

TABLE 2.

Males. Intermittent Starvation.

Number of animals.	Age in weeks.	Weight in gm.	Probable error of mean. + or —	Number of animals.	Age in weeks.	Weight in gm.	Probable error of mean. + or —
23	5	8·89	·21	23	9	13·39	·37
23	6	9·27	·26	23	10	14·96	·38
23	7	10·50	·29	23	11	16·19	·42
23	8	11·81	·30	23	12	17·30	·41

TABLE 2 (continued).

Males. Intermittent Starvation.

Number of animals.	Age in weeks.	Weight in gm.	Probable error of mean. + or −
22	13	18·59	·38
22	14	19·66	·33
22	15	20·25	·35
22	16	21·14	·35
22	17	22·27	·36
22	18	22·50	·36
22	18	22·98	·39
22	20	23·68	·33
22	21	24·07	·30
22	22	24·57	·29
22	23	25·34	·36
22	24	25·91	·29
22	25	26·25	·28
22	26	26·36	·32
22	27	26·61	·37
22	28	26·57	·32
22	29	26·77	·34
22	30	26·59	·34
22	32	26·57	·39
22	34	27·73	·47
22	34	27·73	·47
22	36	28·66	·46
22	38	28·82	·52
22	40	28·98	·48
21	42	29·62	·39
21	44	30·02	·36
21	46	30·43	·45
21	48	30·98	·40
21	50	30·93	·37
21	52	31·12	·40
21	54	31·57	·42
21	56	31·76	·44
21	58	31·74	·40
20	60	31·72	·42
20	62	32·05	·41
20	64	31·87	·42
20	66	32·07	·44
19	68	32·00	·42
19	70	31·76	·41
19	72	31·68	·42
19	74	31·39	·44
19	76	30·87	·43
19	78	30·47	·49
19	80	30·29	·51
19	82	30·32	·58
19	84	29·95	·67
18	86	30·11	·68
17	88	30·00	·73
16	90	29·50	·96
15	92	30·16	·98
14	94	31·39	·51
13	96	31·15	·41
13	98	30·69	·46
13	100	31·27	·67
12	102	30·79	·60
12	104	31·58	·66
12	106	30·62	·60
11	108	29·64	·59
11	110	28·50	1·05
9	112	29·94	·79
8	114	30·19	·93
8	116	29·19	·91
7	118	30·43	1·04
7	120	29·93	1·20
6	122	29·58	1·17
5	124	30·10	·70
5	126	29·50	·84
5	128	29·60	·94
5	130	28·70	·82
5	132	28·50	1·36
4	134	27·62	—
2	136	28·75	—
2	138	27·25	—
2	140	27·25	—
1	142	28·00	—
1	144	26·00	—
1	146	25·00	—

TABLE 3.

Males. Intermittent Starvation + Vegetable Nucleic Acid.

Number of animals.	Age in weeks.	Weight in gm.	Probable error of mean. + or −
24	5	9·10	·17
24	6	8·85	·18
24	7	9·71	·26
24	8	11·37	·30
24	9	12·48	·36
24	10	13·89	·39
24	11	15·33	·37
24	12	16·31	·42
23	13	17·69	·39
23	14	18·69	·41
23	15	19·83	·42
23	16	20·52	·47

TABLE 3 (continued).

Males. Intermittent Starvation + Vegetable Nucleic Acid.

Number of animals.	Age in weeks.	Weight in gm.	Probable error of mean. + or −	Number of animals.	Age in weeks.	Weight in gm.	Probable error of mean. + or −
23	17	21·76	·43	22	72	31·27	·62
23	18	22·17	·41	21	74	31·76	·47
23	19	22·50	·44	21	76	31·14	·54
23	20	23·00	·42	20	80	30·60	·55
23	21	23·89	·40	19	82	30·76	·58
23	22	24·48	·47	19	84	30·34	·61
23	23	25·04	·43	19	86	30·47	·63
23	24	25·39	·42	19	88	30·26	·68
23	25	25·63	·39	19	90	29·89	·69
23	26	26·00	·43	18	92	30·11	·72
23	27	26·17	·39	18	94	30·25	·71
23	28	26·32	·43	18	96	29·63	·87
23	29	26·22	·42	17	98	30·76	·78
23	30	26·00	·48	16	100	30·93	·80
23	32	26·91	·51	15	102	31·13	·80
23	34	27·46	·51	14	104	31·25	·87
23	36	28·11	·54	14	106	31·50	·84
23	38	28·63	·49	12	108	32·96	·76
23	40	29·00	·56	11	110	31·55	·73
23	42	29·15	·59	10	112	31·75	·92
23	44	29·30	·53	10	114	31·40	·88
23	46	29·69	·52	10	116	31·40	·85
23	48	29·69	·57	10	118	30·80	1·00
23	50	29·95	·51	10	120	29·85	1·17
23	52	30·00	·53	8	122	28·94	1·24
23	54	30·69	·54	7	124	29·30	1·55
23	56	30·95	·54	6	126	29·41	1·23
23	58	31·15	·59	6	128	27·66	1·23
23	60	31·43	·60	5	130	27·20	1·26
23	62	31·61	·59	4	132	28·62	—
23	64	31·24	·58	3	134	26·83	—
23	66	31·24	·61	2	136	26·00	—
23	68	30·61	·76	1	138	28·50	—
22	70	31·25	·63	1	140	24·50	—

TABLE 4.

Control Females.

Number of animals.	Age in weeks.	Weight in gm.	Probable error of mean. + or −	Number of animals.	Age in weeks.	Weight in gm.	Probable error of mean. + or −
24	5	8·44	·16	24	15	19·22	·21
24	6	10·02	·28	24	16	19·50	·24
24	7	11·79	·26	24	17	19·93	·23
24	8	13·16	·24	24	18	20·20	·22
24	9	14·58	·24	24	19	20·54	·22
24	10	15·81	·27	24	20	21·08	·25
24	11	16·60	·24	24	21	21·31	·23
24	12	17·31	·22	24	22	21·66	·24
24	13	17·89	·22	24	23	22·12	·27
24	14	18·62	·21	24	24	22·43	·26

TABLE 4 (continued).

Control Females.

Number of animals.	Age in weeks.	Weight in gm.	Probable error of mean. + or −	Number of animals.	Age in weeks.	Weight in gm.	Probable error of mean. + or −
24	25	22·81	·27	21	82	27·09	·70
24	26	22·87	·27	21	84	26·73	·71
24	27	23·12	·29	21	86	26·80	·74
24	28	23·37	·29	21	88	26·45	·73
24	29	23·27	·28	21	90	25·45	·82
24	30	23·54	·32	19	92	26·55	·80
24	32	23·70	·36	18	94	26·50	·81
24	34	24·27	·36	18	96	25·72	·85
24	36	24·93	·37	16	98	26·47	·95
24	38	24·81	·38	16	100	26·87	·89
24	40	25·02	·43	14	102	26·78	·93
24	42	25·35	·42	14	104	26·53	·89
24	44	25·85	·44	13	106	26·00	·96
24	46	26·16	·47	12	108	26·58	·96
23	48	26·43	·57	12	110	26·87	1·00
23	50	26·54	·55	12	112	26·96	1·11
23	52	27·02	·52	12	114	26·46	1·05
23	54	27·56	·57	12	116	26·00	1·10
23	56	27·73	·59	12	118	25·71	·95
23	58	27·84	·65	11	120	23·95	1·06
23	60	27·91	·75	9	122	24·83	1·13
23	62	28·56	·73	9	124	24·00	1·12
23	64	28·80	·77	7	126	23·21	1·43
23	66	28·95	·79	5	128	24·30	1·37
23	68	29·22	·79	3	130	23·16	—
23	70	29·00	·77	3	132	23·33	—
23	72	28·54	·73	3	134	23·16	—
23	74	28·67	·83	2	136	19·75	—
23	76	28·65	·79	1	138	17·50	—
23	78	27·90	·67	1	140	15·50	—
21	80	27·50	·72				

TABLE 5.

Intermittent Starvation. Females.

Number of animals.	Age in weeks.	Weight in gm.	Probable error of mean. + or −	Number of animals.	Age in weeks.	Weight in gm.	Probable error of mean. + or −
23	5	8·83	·19	23	19	21·59	·30
23	6	9·30	·23	23	20	21·87	·30
23	7	10·78	·30	23	21	22·39	·27
23	8	12·30	·27	23	22	22·67	·30
23	9	13·48	·35	23	23	22·78	·31
23	10	14·72	·33	23	24	23·22	·33
23	11	16·04	·34	23	25	23·35	·28
23	12	16·65	·33	23	26	23·46	·28
23	13	17·67	·37	23	27	23·61	·26
23	14	18·56	·37	23	28	23·61	·27
23	15	19·48	·35	23	29	23·76	·31
23	16	19·89	·32	23	30	23·89	·30
23	17	20·87	·33	23	32	24·26	·32
23	18	21·09	·32	23	34	25·17	·33

TABLE 5 (continued).

Intermittent Starvation. Females.

Number of animals.	Age in weeks.	Weight in gm.	Probable error of mean. + or —	Number of animals.	Age in weeks.	Weight in gm.	Probable error of mean. + or —
23	36	25·50	·32	17	98	29·05	·55
23	38	25·67	·32	15	100	28·53	·67
23	40	25·74	·33	15	102	28·83	·66
23	42	26·17	·37	15	104	28·07	·57
23	44	26·69	·33	15	106	28·10	·65
23	46	27·39	·37	14	108	28·50	·66
23	48	27·65	·40	14	110	28·32	·66
23	50	27·46	·39	14	112	28·18	·70
23	52	27·54	·37	14	114	27·43	·85
23	54	28·02	·40	13	116	28·07	·67
23	56	27·83	·44	13	118	28·19	·65
22	58	28·36	·43	13	120	28·15	·65
22	60	28·54	·41	13	122	26·54	·62
22	62	28·41	·40	13	124	25·69	·78
22	64	28·82	·43	13	126	24·38	·73
22	66	28·84	·38	10	128	25·25	·97
22	68	28·93	·44	9	130	25·61	·63
22	70	28·75	·45	7	132	25·57	·59
22	72	28·73	·41	7	134	23·42	·74
22	74	28·59	·44	6	136	22·50	1·01
22	76	28·14	·42	5	138	22·80	·73
21	78	27·69	·44	4	140	21·87	—
20	80	28·25	·41	2	142	20·75	—
20	82	28·17	·50	2	144	21·00	—
20	84	27·92	·45	1	146	21·50	—
20	86	27·82	·47	1	148	22·50	—
20	88	27·80	·53	1	150	22·00	—
20	90	27·77	·54	1	152	22·00	—
20	92	27·57	·57	1	154	24·00	—
18	94	28·22	·55	1	156	23·50	—
18	96	27·94	·63				

TABLE 6.

Intermittent Starvation + Vegetable Nucleic Acid. Females.

Number of animals.	Age in weeks.	Weight in gm.	Probable error of mean. + or —	Number of animals.	Age in weeks.	Weight in gm.	Probable error of mean. + or —
24	5	8·89	·21	23	18	20·80	·32
24	6	8·98	·20	23	19	21·37	·28
24	7	10·12	·19	23	20	22·00	·30
24	8	11·25	·31	23	21	22·37	·29
24	9	12·29	·33	23	22	22·69	·29
24	10	13·54	·36	23	23	22·89	·30
23	11	14·80	·39	23	24	23·56	·27
23	12	16·06	·35	23	25	23·37	·30
23	13	16·78	·36	23	26	23·50	·29
23	14	17·87	·34	23	27	23·41	·30
23	15	18·83	·29	23	28	23·80	·24
23	16	19·50	·29	23	29	23·96	·28
23	17	20·15	·30	23	30	24·09	·31

TABLE 6 (continued).

Intermittent Starvation + Vegetable Nucleic Acid. Females.

Number of animals.	Age in weeks.	Weight in gm.	Probable error of mean. + or −	Number of animals.	Age in weeks.	Weight in gm.	Probable error of mean. + or −
23	32	24·56	·35	13	98	26·65	1·00
23	34	25·28	·33	12	100	27·16	·83
23	36	25·76	·30	11	102	27·86	·76
23	38	25·67	·32	11	104	27·95	·77
23	40	26·02	·31	10	106	27·80	·82
23	42	26·61	·35	10	108	26·70	1·13
23	44	27·33	·35	8	110	28·25	1·12
23	46	27·30	·34	8	112	28·18	·99
23	48	27·96	·33	8	114	28·87	1·26
23	50	27·67	·35	6	116	27·91	1·15
23	52	28·00	·35	6	118	27·74	1·11
23	54	27·93	·40	6	120	28·20	1·35
23	56	28·28	·39	5	122	27·40	1·46
23	58	28·39	·40	4	124	28·50	—
23	60	28·48	·37	4	126	28·22	—
23	62	28·80	·37	4	128	27·87	—
23	64	28·69	·42	4	130	27·87	—
23	66	28·93	·40	4	132	27·50	—
23	68	28·72	·39	4	134	26·66	—
23	70	28·65	·44	3	136	24·83	—
23	72	28·35	·41	3	138	25·50	—
23	74	28·33	·44	3	140	25·16	—
23	76	28·00	·52	2	142	23·25	—
23	78	27·74	·48	2	144	23·75	—
22	80	27·73	·53	2	146	21·50	—
22	82	27·48	·55	2	148	20·50	—
22	84	27·16	·55	1	150	28·00	—
22	86	26·79	·60	1	152	28·50	—
21	88	26·74	·69	1	154	28·00	—
20	90	26·87	·64	1	156	27·50	—
20	92	26·32	·71	1	158	26·50	—
17	94	26·00	·76	1	160	26·00	—
14	96	26·03	·96	1	162	26·00	—

TABLE 7.

Intermittent Starvation Experiment. Mortality Statistics.

	Male Animals.				Female Animals.			
Age in Days.	Control.	Intermittent Starvation.	Intermittent Starvation + Nucleic Acid.		Age in Days.	Control.	Intermittent Starvation.	Intermittent Starvation + Nucleic Acid.
	Per cent. Survivors.					Per cent. Survivors.		
200	100	100	100		600	82·6	81·8	82·6
250	100	100	100		650	73·9	68·2	78·3
300	95·6	95·5	100		700	69·5	59·1	69·6
350	91·3	95·5	100		750	60·8	50·0	52·2
400	91·3	95·5	100		800	43·4	36·4	43·5
450	86·9	90·9	100		850	34·7	27·3	34·8
500	82·6	86·4	95·7		900	17·4	22·7	26·1
550	82·6	86·4	87·0		950	8·7	9·1	8·6

TABLE 7 (continued).

Intermittent Starvation Experiment. Mortality Statistics.

Male Animals.				Male Animals.			
Age in Days.	Control.	Intermittent Starvation.	Intermittent Starvation + Nucleic Acid.	Age in Days.	Control.	Intermittent Starvation.	Intermittent Starvation + Nucleic Acid.
	Per cent. Survivors.				Per cent. Survivors.		
1,000	8·7	4·5	—	600	87·5	87·0	95·7
1,050	—	—	—	650	79·2	87·0	87·0
1,100	—	—	—	700	66·7	65·2	52·2
1,150	—	—	—	750	50·0	60·9	43·5
1,200	—	—	—	800	50·0	60·9	34·8
200	100	100	100	850	37·5	56·5	21·7
250	100	100	100	900	20·8	44·0	17·4
300	100	100	100	950	8·3	26·1	13·0
350	95·8	100	100	1,000	—	8·7	8·7
400	95·8	95·7	100	1,050	—	4·3	4·3
450	95·8	95·7	100	1,100	—	—	4·3
500	95·8	95·7	100	1,150	—	—	—
550	91·7	91·3	100	1,200	—	—	—

Mean Duration of Life.

Males	Control	712 days.	Probable error	27·27 days.
	Intermittent Starvation	745 „	„ „	26·75 „
	Intermittent Starvation + Nucleic Acid	769 „	„ „	21·00 „
Females	Control	773 days.	Probable error	21·00 days.
	Intermittent Starvation	819 „	„ „	24·7 „
	Intermittent Starvation + Nucleic Acid	763 „	„ „	20·5 „

P. 40, Table 7. Both sets of mortality statistics refer to *male* animals.

P. 41, Table 7. Data subsequent to 1,200 days in the first column refer to *female* animals.

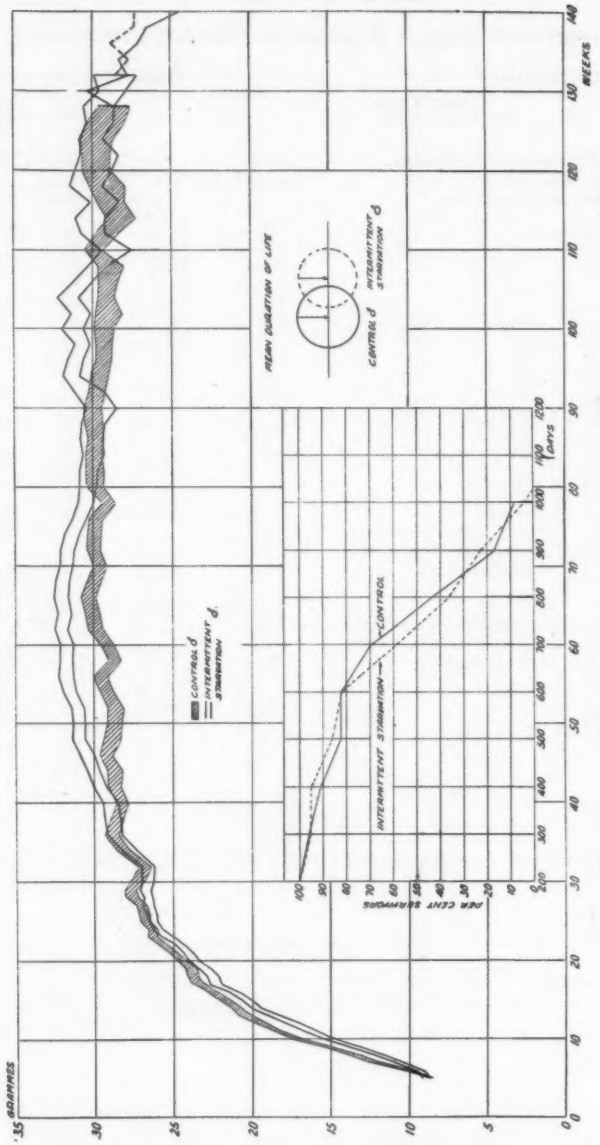

Fig. 1. The growth and longevity of a group of male mice that were subjected to intermittent starvation two days in each week throughout the complete life cycle is contrasted with that of normal male mice which were continually offered the same diet, *ad lib.*

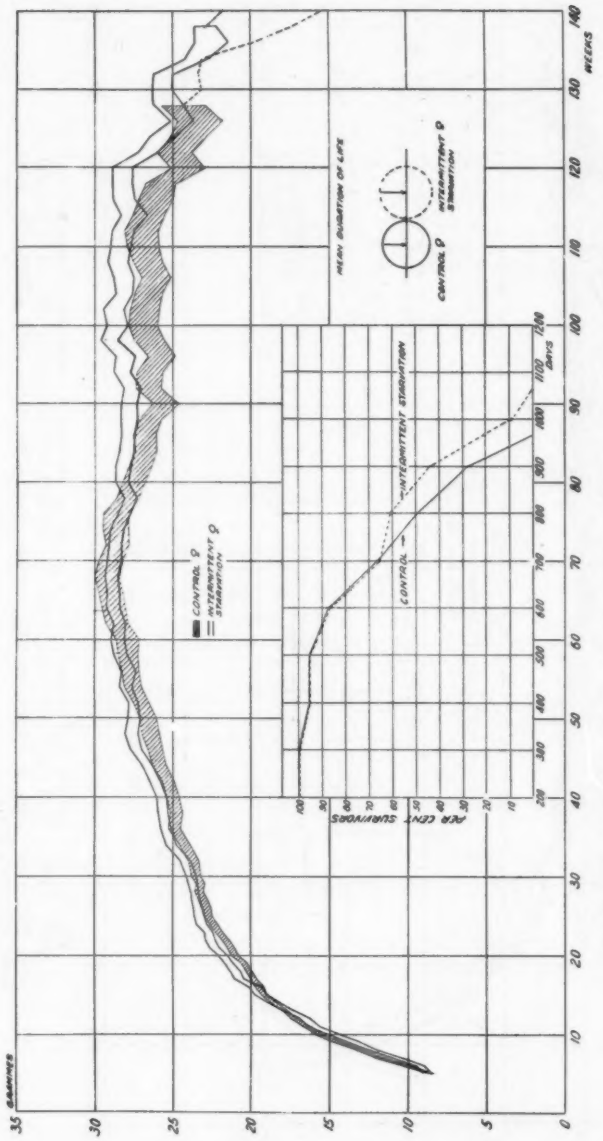

Fig. 2. The growth and longevity of a group of female mice that were subjected to intermittent starvation two days in each week throughout the complete life cycle is contrasted with that of normal female mice which were continually offered the same diet, *ad lib.*

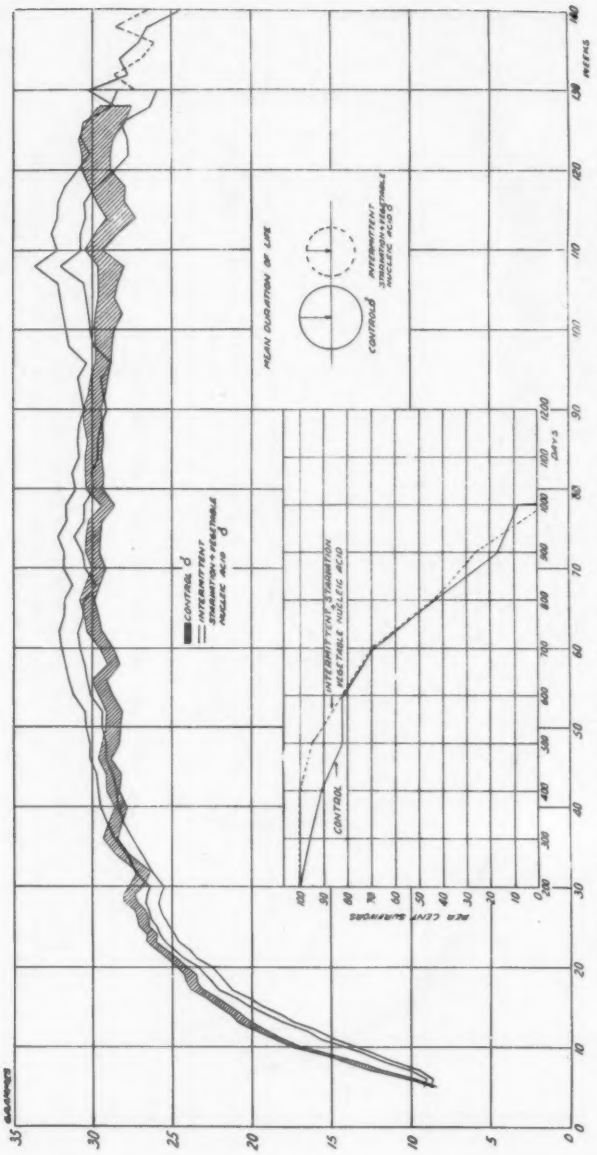

Fig. 3. The growth and longevity of a group of male mice receiving 25 mg. per day of yeast nucleic acid that were subjected to intermittent starvation two days in each week throughout life, is contrasted with normal male mice which were offered the same basal diet each day.

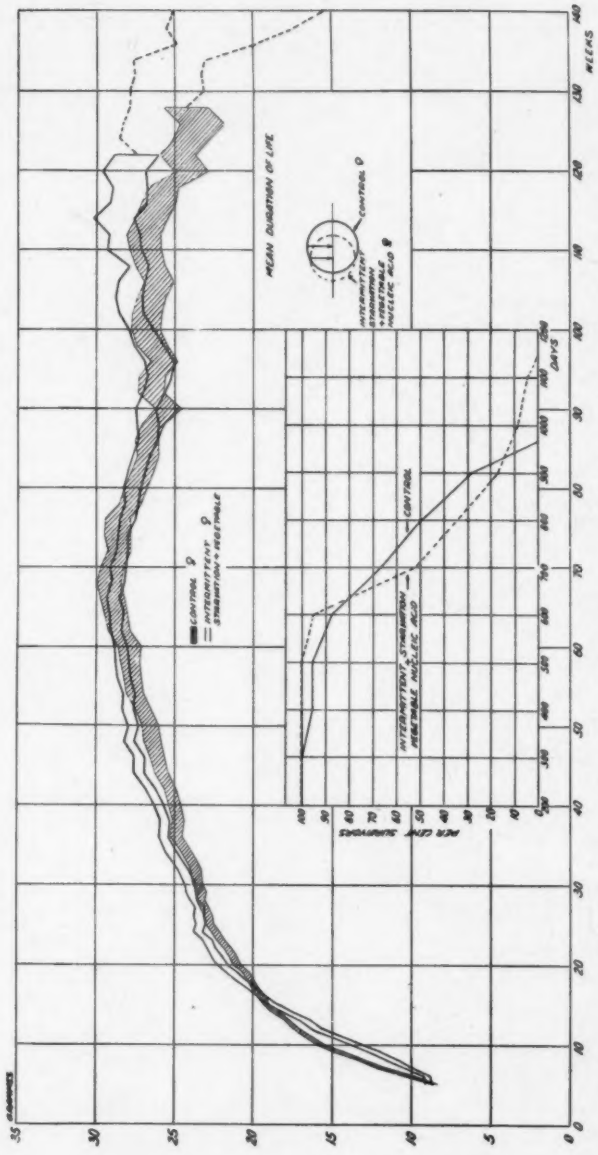

Fig. 4. The growth and longevity of a group of female mice receiving 25 mg. per day of yeast nucleic acid that were subjected to intermittent starvation two days in each week throughout life, is contracted with normal female mice which were offered the same basal diet each day.